EFFICACY OF PLATELET RICH FIBRIN ASSISTED WITH AND WITHOUT LASER APPLICATION IN THE TREATMENT OF PERIODONTAL INTRABONY DEFECTS – A CLINICO RADIOGRAPHIC STUDY

A Dissertation submitted in

partial fulfillment of the requirements

for the degree of

MASTER OF DENTAL SURGERY

BRANCH – II

PERIODONTOLOGY

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI – 600032

2014 – 2017

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled **"Efficacy Of Platelet Rich Fibrin Assisted With And Without Laser Application In The Treatment Of Periodontal Intrabony Defects – A Clinicoradiographic Study"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.C.S.PRABHAHAR., M.D.S., Professor, Head of the Department,** Department Of Periodontology, Best Dental Science College, Madurai – 625104.

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The mediocre teacher tells. The good teacher explains. The superior teacher demonstrates. The great teacher inspires.

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DECLARATION

TITLE OF DISSERTATION	EFFICACY OF PLATELET RICH FIBRIN ASSISTED WITH AND WITHOUT LASER APPLICATION IN THE TREATMENT OF PERIODONTAL INTRABONY DEFECTS – A CLINICO RADIOGRAPHIC STUDY
PLACE OF STUDY	BEST DENTAL SCIENCE COLLEGE, MADURAI – 625104.
DURATION OF THE COURSE	3 YEARS
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This agreement herein after the "Agreement" is entered into on this day 30th Dec 2016. between the Best Dental Science College represented by its **Principal** having address at Best Dental Science College, Madurai – 625104, (hereafter referred to as, 'the College')

And

Mr. Dr.C.S.PRABHAHAR, M.D.S., aged 44 years working as **Professor and HOD** in Department of Periodontology at the College, having residence address at 9/3 Vallalar Street, Alagappapuram, Karaikudi – 630 002, Sivagangai district.

(herein after referred to as the 'Principal Investigator')

And

Mr. Dr.S.P.BRINDHA DEVI aged 27 years currently studying as **Post Graduate student** in Department of Periodontology, Best Dental College, Madurai- 625104 (herein after referred to as the 'PG/Research student and co-investigator')

Whereas the PG/Research student as part of his curriculum undertakes to research on **"EFFICACY OF PLATELET RICH FIBRIN ASSISTED WITH AND WITHOUT LASER APPLICATION IN THE TREATMENT OF PERIODONTAL INTRABONY DEFECTS – A CLINICO RADIOGRAPHIC STUDY"** for which purpose PG/Principal Investigator shall act as Principal Investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG/Research student as to the extent possible as a Co-investigator.

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Place: MADURAI

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LIST OF ABBREVIATIONS USED

FGF	Fibroblast growth factors
PDGF	Platelet-derived growth factor
IGFs	Insulin- like growth factors
TGFs	Transforming growth factors
EGF	Epidermal growth factor
PRF	Platelet rich fibrin
GaAlAs	Gallium-aluminum-arsenide
PPD	Probing pocket depth
CAL	Clinical attachment level
PDT	Photodynamic therapy
MB	Methylene blue
Er: YAG	Erbium-doped yttrium aluminum garnet laser
SRP	Scaling and root planing
BOP	Bleeding on probing

GI	Gingival Index
PI	Plaque Index
LLLT	Low-level laser therapy
OPG	Osteoprotegerin
p-ERK	Extracellular signal related protein kinase
ALP	Alkaline phosphatase
BPBM	Bovine porous bone mineral
GTR	Guided tissue regeneration
GAgP	Generalized aggressive periodontitis
PRP	Platelet rich plasma
OFD	Open flap debridement
DFDBA	Demineralized freeze dried bone allograft
RUNX2	Runt-related transcription factor 2
GL	Gingival level
MGI	Modified gingival index
DL	Diode Laser

β-TCP	β – tricalcium phosphate
MF	Metformin
IBD	Intrabony defects
mSBI	Modified sulcus bleeding index
GML	Gingival marginal level
RAL	Relative attachment level
CP	Chronic periodontitis
EMD	Enamel matrix derivative
GR	Gingival recession
DD	Defect depth
DW	Defect width
DA	Defect angle
hDPSCs	Human dental pulp stem cells
ATV	Atorvastatin
ALN	Alendronate
RSV	Rosuvastatin

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Introduction

INTRODUCTION

Periodontitis is a disease of the periodontium characterized by the irreversible loss of connective tissue attachment and supporting alveolar bone. Bone tissue maintains its homeostasis by bone formation and resorption. This equilibrium is disrupted when resorption exceeds formation as in the case of periodontal disease. Bone resorption results in the alteration of its normal morphologic features. The hallmark of periodontal disease are destruction of connective tissues and the bone loss which is generally considered to represent the anatomical sequela to the apical spread of periodontitis.[1]

According to Pritchard (1965), osseous defects caused by periodontal disease can be interproximal craters, inconsistent margins, hemisepta, furca invasions, intrabony defects and combinations of those defects.[2] Initially, Periodontal therapy is directed at disease prevention, slowing or arresting disease progression, regenerating lost periodontium, and maintaining achieved therapeutic objectives.[3]

The ultimate goal of periodontal therapy has been the regeneration of the supporting tissues which is lost as a consequence of inflammatory periodontal disease. Over the years, several reports have suggested or claimed the achievement of periodontal regeneration as the result of various modes of therapy. Treatment has included root planing and soft tissue curettage and various types of flap procedures, often in combination with the placement of bone grafts or bone substitutes into the defects. In most of these reports, such clinical parameters as probing pocket depth, probing attachment level, radiographic analysis and surgical re-entry procedures have been used to evaluate the result of therapy.[4]

In order for periodontal regeneration to occur, progenitor periodontal ligament cells must migrate to the denuded root surface, attach to it, proliferate and mature into an organized and functional fibrous attachment apparatus and progenitor bone cells must also migrate, proliferate and mature in conjunction with the regenerating periodontal ligament. Significant advances have been made in the last decade in understanding the factors controlling the migration, attachment and proliferation of cells which are mainly responsible for regeneration to occur. Key regulators of these biological events are a group of naturally occurring molecules known as polypeptide growth factors in conjunction with certain matrix proteins. Of these, the fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), insulin- like growth factors (IGFs), transforming growth factors (TGFs), epidermal growth factor (EGF) and certain attachment proteins appear to have an important role in periodontal wound healing.[5]

Platelet rich fibrin is a second-generation platelet concentrate for surgical use. Platelet rich fibrin is an immune and platelet concentrate collecting on a single fibrin membrane all the constituents of a blood sample favorable to healing and immunity.[6]

PRF consists of an assembly of cytokines, glycanic chains, and structural glycoproteins enmeshed within a slowly polymerized fibrin network.[7] The three dimensional structure of the matrix resembles that of physiologic fibrin and the enmeshed cytokines influence the extracellular matrix which allows migration, division and phenotypic change of endothelial cells, thus leading to angiogenesis.[7] It also has a dense fibrin network with leukocytes, cytokines, structural glycoproteins, and also GFs and the leukocytes that are concentrated in PRF scaffold play an important role in GF release, immune regulation, anti - infectious activities, and matrix remodelling during wound healing. The slow

polymerization mode of PRF and cicatricial capacity create a physiologic architecture favorable for wound healing.[8] PRF, as a physiologic fibrin matrix, serves as a net to stem cells, especially when an accelerated angiogenesis develops in the fibrin membrane. This aspect of particular interest in the case of wide osseous defects.

Diode is a solid active medium laser, manufactured from semiconductor crystals using some combination of aluminum or indium, gallium, and arsenic. All of the diode wavelengths are highly absorbed by pigmented tissue and are deeply penetrating.[9] The diode is expected to have a disinfecting thermal effect on bacteria that is basically limited to root surface and it also reveals a bactericidal effect especially on pathogenic bacteria like Aggregatibacter actinomycetemcomitans.[10] Gallium-aluminum-arsenide (GaAlAs) laser, increased both fibroblast proliferation and accelerated formation of bone matrix.[11]

The complete removal of bacteria and their toxins from periodontal pockets is not always achieved with conventional mechanical treatment. It can be used as an adjunctive therapy for periodontal disease may improve tissue healing by bactericidal and detoxification effects.[12]

The present study was undertaken to clinically and radiographically evaluate the efficacy of platelet rich fibrin (PRF) assisted with and without laser application in the treatment of periodontal intrabony defects.

Aim and Objectives

AIM AND OBJECTIVES

AIM:

To compare the efficacy of Platelet rich fibrin assisted with and without laser application in the treatment of periodontal intrabony defects.

OBJECTIVES:

1. To assess the clinical parameters like Plaque index, Gingival index, Probing pocket depth (PPD), Clinical attachment level (CAL) at baseline and following surgical therapy at 1 month, 3months & 6 months post operatively.

2. To determine the bone level radiographically at baseline & 6 months post operatively.

Review of Literature

GENERAL REVIEW

Periodontitis is primarily plaque induced inflammation involving and destroying the supporting alveolar bone and periodontal ligament. Treatment of periodontitis involves mechanical debridement and chemotherapeutic procedures aimed at eliminating the pathological bacterial flora responsible for the inflammation. This treatment leaves a defect characterized clinically by bone and periodontal ligament loss, gingival pockets and recession of the gingival margin. Periodontal surgery is primarily directed at resolving these defects, either by repair or regeneration.[13]

Since treatment progress is limited in advanced periodontitis lesions following cause-related therapy, periodontal surgical procedures have been advocated as integral part of periodontal therapy for many decades.

Depending on the objectives to be achieved, various surgical techniques are used. Such objectives include: the surgical elimination of pockets, access to the root surfaces for open debridement, periodontal regeneration characterized by the formation of new root cementum, new periodontal fibers and new alveolar bone and healing of bony defects by filling in with new bone.

Intrabony defects:

An intrabony defect is defined as a ‘‘Periodontal defect within the bone surrounded by one, two or three bony walls or a combination thereof’. (According to the glossary of terms of the American Academy of Periodontology)

Intrabony defects are usually classified according to the criteria presented by Goldman & Cohen as one-wall intrabony defects: defects limited by one osseus wall and the tooth surface; two-wall intrabony defects: defects limited by two osseous walls and the tooth surface; and three-wall intrabony defects: defects limited by three osseous walls and the tooth surface.[14]

THE CONCEPT OF PERIODONTAL RECONSTRUCTION

According to **AH Melcher in 1976**, regeneration of periodontal ligament is of prime importance as it provides continuity between the alveolar bone and the cementum and also it apparently contains cells that can synthesize and remodel the three connective tissues of the alveolar part of the periodontium.[15]

Following flap procedure, if gingival epithelium migrates along the connective tissue adjacent to a treated root, healing will occur by a long junctional epithelium and new attachment will not occur. If gingival connective tissue gains access to the root surface, there will be a connective tissue adhesion. When bone is in contact with the root, there is the potential for ankylosis and root resorption. Periosteum and undifferentiated cells within the marrow spaces may also contribute to the regenerative process. The periodontal ligament contains the cell populations that are capable of contributing to new attachment.[16]

Tissue engineering and the periodontium:

Tissue engineering is a contemporary area of applied biomedical research aimed at developing procedures and biomaterials for the fabrication of new tissues to replace damaged tissues and is based on principles of cell biology, developmental biology, and

biomaterials science. Preliminary studies have indicated that periodontal ligament and bone cells can be transplanted into periodontal sites with no adverse immunological or inflammatory consequences. Thus an emerging paradigm of "biological solutions" for "biological problems" is appearing in both clinical dentistry and medicine.

Recent advances in growth factor biology and biodegradable polymer technology have set the stage for successful tissue engineering of cartilage, bone and related tissues of which the periodontium could be considered a prime candidate for such procedures. Through the provision of a prefabricated three-dimensional structure with the appropriate instructive messages via a variety of growth and differentiation factors incorporated, it will be possible to overcome many of the limitations associated with conventional regenerative technologies.[17]

PLATELET RICH FIBRIN:

Platelet rich fibrin is a second generation therapeutic platelet concentrate, the first being the platelet rich plasma. PRF was first described by Choukroun et al. in the year 2001. The use of this platelet concentrate during reconstructive surgery helps in several ways.

First, the PRF membrane protects and maintains the grafted material and its fragments serving as a biological connector between the bone graft materials. Second, the integration of this fibrin network into the regenerative site helps in cellular migration, mainly for endothelial cells required for the neo-angiogenesis, vascularization and survival of the graft. Third, the platelet cytokines such as PDGF, TGF- β, IGF-1 are gradually released as the fibrin matrix is resorbed, and hence it creates a perpetual

process of healing. Lastly, the presence of leukocytes and cytokines in the fibrin meshwork plays a significant role in the self-regulation of the inflammatory and infectious phenomena within the grafted material.[18]

Important growth factors used for regeneration purposes are:

- Fibroblast growth factor
- Transforming growth factor-β
- Insulin like growth factor
- Platelet derived growth factor

Growth factors are biological mediators which regulate connective tissue cell migration, proliferation and synthesis of proteins and other components of the extracellular matrix.[19]

In PRF growth factors are slowly released for 7 days and these growth factors are mainly responsible for the healing and regeneration potential of PRF.

TRANSFORMING GROWTH FACTOR-β:

Primary effect of TGF- β on mesenchymal cells is to aid in matrix synthesis.

During the early stages of bone formation, the action of transforming growth factor-β is to recruit and stimulate osteoprogenitor cells to proliferate, providing a pool of early osteoblasts. In contrast, during later phases of osteoblast differentiation, transforming growth factor-β blocks differentiation and mineralization.[20] Regulates its activity through the stimulation of the smad pathway, following which it upregulates the cbfal. These effects are sometimes aided by cross talk through the MAPK pathway.

Advantages:

- In periodontal defects – stimulates two important tissues of the periodontium, the alveolar bone and the periodontal ligament.
- Causes increased differentiation of the stromal cells of the bone as well as undifferentiated mesenchymal cells of the periodontium.
- Antiepithelial in nature - cause apoptosis of the epithelial cells and downregulate epithelial proliferation and differentiation. Therefore, the presence of TGF- β retards epithelial proliferation and thus prevents the formation of long junctional epithelium.
- Thus, TGF- β delivery is beneficial in inducing differentiation of the cells of the bone and periodontal ligament, at the same time, preventing the growth of the unwanted epithelial cells.

PLATELET DERIVED GROWTH FACTOR:

PDGF has a family of four members that have been classified into PDGF A, B, C and D. It is generally thought to be chemotactic, proliferative and capable of inducing differentiation of the mesenchymal cells and the predominant effect is to induce differentiation of the stromal cells to form osteoblasts.

Its isoforms have a strong chemotactic effect on osteoblasts and other connective tissue cells, and may act to recruit mesenchymal cells during bone development and remodeling.[20] Similarly, the periodontal ligament fibroblasts are also differentiated following application of PDGF. PDGF-D - initial mineralization activator and thought to be important for the early matrix synthesis and mineralization. PDGF-A and C are also

thought to be positive inducers of differentiation of osteoblasts. Hence, these growth factors are one of the growth factors that have been widely used in the regeneration of vertical osseous defects and they have been used both in their naturally occurring and recombinant forms.

INSULIN LIKE GROWTH FACTOR:

IGF is a mitogenic growth factor that belongs to a family of proteins that bear a strong structural homology to the insulin molecule and is capable of expanding the stromal cell population in the bone forming cells. Two insulin-like growth factors have been identified– insulin-like growth factor-1 and insulin-like growth factor-2 – both of which are found in high concentration in serum.

Insulin-like growth factors increase proliferation and play a major role in stimulating mature osteoblast function.[20] At the molecular level, insulin-like growth factor-1 upregulates the osteoblast-associated transcription factor, osterix, but not Cbfa1/Runx2.

IGF also has the potential to be used in concert with other growth factors so as to optimize their effects, e.g. IGF may be used in concert with PDGF so as to maximize the benefits of both growth factors in the wound space.

Diode laser:

Diode is a solid active medium laser, manufactured from semiconductor crystals using some combination of aluminum or indium, gallium, and arsenic and depending on wavelength either aluminum or indium is used. All of the diode wavelengths are highly absorbed by pigmented tissue and are deeply penetrating and hence it is an excellent soft

tissue surgical laser and is indicated for cutting and coagulating gingiva and mucosa and for sulcular debridement.[21]

Low-level laser therapy is provided by semiconductor instruments emitting visible and invisible near infrared light energy at powers significantly below any surgical interactive threshold. They can provide biostimulation of osteoblast and helps in mineralization of bone.[21, 22]

LLLT may act as an inducer factor for osteogenesis. Studies suggests that LLLT would improve bone matrix production due to improved vascularization and anti-inflammatory effect and these aspects would increase both the release of mediators & micro vascularization which in turn would accelerate would healing. IGF also has the potential to be used in concert with other growth factors so as to optimize their effects, e.g. IGF may be used in concert with PDGF so as to maximize the benefits of both growth factors in the wound space.

LLLT devices includes:

- acceleration of wound healing
- enhanced remodelling and repair of bone
- restoration of normal neural function following injury
- normalization of abnormal hormonal function
- pain attenuation
- stimulation of endorphin release and
- modulation of the immune system.

LLLT has also been shown to stimulate the production of basic fibroblast growth factor (bFGF), which supports fibroblast proliferation and differentiation. Fibroblasts irradiated

with lowdose LLLT show both increased cell proliferation and also enhanced production of bFGF, while high dose suppresses both parameters, indicating a causal relationship between autocrine production of bFGF from fibroblasts and proliferation. Studies shows that it causes increased accumulation of calcium and accelerates calcification in vitro.[23]

Andreas Moritz et al (1998)[24] examined the long-term effects of diode laser therapy on periodontal pockets with regard to its bactericidal abilities and the improvement of periodontal condition. Fifty patients were selected and all teeth were treated with the diode laser and the control group received the same treatment but instead of laser therapy were rinsed with H_2O_2. Microbiologic samples were collected before and 6 months after treatment. Results showed that bacterial reduction and index of bleeding was better in group treated with diode laser therapy. Hence they concluded that the diode laser reveals a bactericidal effect and also helps to reduce inflammation in the periodontal pockets in addition to scaling.

Goharkhay et al (1999)[25] determined incision characteristics and soft-tissue damage resulting from standardized incisions using a wide range of laser modes and parameters of a diode laser at 810 nm. Histologic examinations were performed to verify vertical and horizontal tissue damage as well as incision depth and width. Results showed that incision depth and width correlated with average powers, but not with laser parameters or the used tips. No laser damage was visible to the naked eye in bone underlying the incisions in the range between 0.5–4.5 W. They concluded that there was remarkable cutting ability and the tolerable damage zone clearly showed that the diode laser was very

effective and because of its excellent coagulation ability, which was used as an alternative in soft-tissue surgery of the oral cavity.

You Chan et al (2003)[26] attempted to clarify that whether the bactericidal effects of photodynamic therapy (PDT) was wavelength or dose-dependent. Cultures of Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, and Streptococcus sanguis, were exposed to a He-Ne laser with a 30 mW power output, a 100 mW diode laser at 665 nm, or a 100 mW diode laser at 830 nm, in the presence or absence of methylene blue (MB) as a photosensitiser. The results indicated that exposure to the 100 mW laser light could eliminate up to 40% of bacteria on average. Best photodynamic therapy response was achieved with a 60s exposure to the 665 nm wavelength diode laser in the presence photosensitiser. Approximately 95% of A. actinomycetemcomitans and F. nucleatum, and 99–100% of the black-pigmented bacteria like P.gingivalis and P.intermedia and S.sanguis were eliminated.

Theodoro et al (2003)[27] compared the effects of diode laser and Er: YAG treatments of the root surface on intrapulpal temperature after scaling and root planing with hand instruments. Fifteen extracted single rooted teeth were scaled and root planed with hand instruments and divided into three groups among which group I lased with Er:YAG laser, 2.94 µm/ 100 mJ/10 Hz/ 30 seconds, group II with diode laser 810nm/ 1.0W/ 0.05 ms/ 30 seconds and group III with diode laser, 810nm/ 1.4W/ 0.05 ms/ 30 seconds. They concluded that the application of Er:YAG and diode lasers at the utilized parameters did

not induce high pulpal temperatures and root surface irregularities were more pronounced after irradiation with an Er:YAG laser than with a diode laser.

Ugo Caruso et al (2008)[28] compared the effectiveness of Diode laser used as an adjunctive therapy of SRP to that of SRP alone for non surgical periodontal treatment in patients with chronic periodontitis. Clinical measurements such as PPD, CAL, BOP, GI, PI were performed before treatment at baseline, after 4 weeks, 8 weeks, 12 weeks and 6 months. Subgingival plaque samples were taken at baseline and after treatment and examined for 8 periopathogens bacteria. The results showed that the additional treatment with diode laser may lead to a slightly improvement of clinical parameters, but there were no significant differences between test and control group in the reduction of periodontopathogens were found.

Ehrenfest et al. (2010)[29] investigated the three - dimensional architecture of Platelet rich fibrin and evaluated the influence of different collection tubes (dry glass or glass coated plastic tubes) and compression procedures (forcible or soft) on the final Platelet rich fibrin architecture. PRF preparation was done in 10 healthy subjects by centrifuging the collected blood at 3000 rpm for 10 minutes. The results revealed that the preparation protocol concentrated most of the platelets and leukocytes from a blood harvest into a single autologous fibrin biomaterial. The study also showed that the type of the test tube (dry or glass coated plastic tubes) and the compression procedures of clot did not influence the architecture of the platelet rich fibrin.

Alice Dias PETRI et al (2010)[30] investigated the effects of low-level laser therapy (LLLT) on human osteoblastic cells grown on titanium (Ti) by using gallium aluminum arsenide (GaAlAs) diode laser. Osteoblastic Cells were exposed to LLLT at 3 J/cm2 (wavelength of 780 nm) at days 3 and 7 and non-irradiated cultures were used as control. Results indicated that LLLT modulates cell responses in a complex way by stimulating osteoblastic differentiation, which suggests benefits on implant osseointegration despite a transient deleterious effect immediately after laser irradiation.

Alireza Fallah (2010)[31] compared the effect of 980 Diode laser plus scaling and root planing (SRP) versus SRP alone in the treatment of chronic periodontitis. The gingival index, probing pocket depth and bleeding on probing were examined at the baseline and after 6 weeks after the start of treatment in 21 sites of each group. Results revealed that both groups showed statistically significant improvements in all parameters but there is significant improvement in laser and SRP group compared to SRP alone. It was concluded that the combination of 980 Diode laser irradiation in the gingival sulcus and SRP, was significantly better as compared to SRP alone.

Thorat MK et al (2011)[32] investigated the clinical and radiological effectiveness of autologous PRF in the treatment of intra-bony defects of chronic periodontitis patients. Thirty-two intra-bony defects were treated either with autologous PRF or a conventional open flap debridement alone. Clinical parameters such as plaque index, sulcus bleeding index, probing depth, clinical attachment level and gingival marginal level were recorded at baseline and 9 months post-operatively. They concluded that there was greater

improvements in parameters at sites treated with PRF than the open flap debridement alone.

Sharma et al. (2011)[33] investigated the effect of Platelet rich fibrin in the treatment of 3 wall intrabony defects. Fifty six intrabony defects in 42 patients were treated with either autologous platelet rich fibrin along with open flap debridement or with open flap debridement alone. Clinical parameters were measured at baseline and 9 months post operatively including probing depth, periodontal attachment level and gingival margin level. Results of the study revealed that significant improvement in clinical parameters and significant defect fill at the sites treated with Platelet rich fibrin compared to sites treated with open flap debridement alone. It was concluded that PRF has excellent properties to enhance periodontal wound healing substantiating the use of PRF in the treatment of intrabony defects.

Chang et al. (2011)[34] investigated the effects of Platelet rich fibrin on human periodontal ligament fibroblast and its application for reconstruction of periodontal intrabony defects. Periodontal ligament fibroblast was obtained from healthy individuals undergoing extraction for orthodontic reasons. The effect of Platelet rich fibrin on periodontal ligament fibroblast was determined by measuring the expression of extracellular signal related protein kinase (p-ERK), osteoprotegerin (OPG) and alkaline phosphatase activity (ALP). The results of the study revealed that Platelet rich fibrin increases the extracellular signal related protein kinase (p-ERK), osteoprotegerin (OPG) and alkaline phosphatase activity (ALP) and it was concluded that Platelet rich fibrin provides benefits for

periodontal regeneration and hence can be used as an effective treatment modality for periodontal intrabony defects.

Sharma et al. (2011)[35] evaluated the effect of Platelet rich fibrin in the treatment of mandibular degree II furcation defects. 18 patients with contralateral buccal Degree II furcation defects were treated either with platelet rich fibrin along with open flap debridement or with open flap debridement alone. Clinical parameters including probing depth, clinical attachment level, gingival margin level and radiographic parameters were evaluated at baseline and 9 months post operatively. The results of the study showed that there was significant improvement in all the clinical and radiological parameters at the sites treated with platelet rich fibrin compared to open flap debridement alone. They concluded that platelet rich fibrin was effective in the regenerative treatment of furcation defects.

Lekovic et al (2011)[36] examined the suitability of autologous PRF as regenerative treatment for periodontal intrabony defects in humans and also examined the ability of Bovine porous bone mineral (BPBM) to augment the regenerative effects exerted by PRF. 17 paired intrabony defects were randomly treated either with PRF or with PRF–BPBM combination and evaluated the pocket depth, attachment level and defect fill by method of surgical re-entry after 6 months. Postsurgical measurements revealed greater improvements in the PRF–BPBM group when compared with the PRF group. It was concluded that BPBM has the ability to augment the effects of PRF.

Abhishek Singh et al (2012)[37] evaluated the efficacy of autologous platelet rich fibrin in soft tissue healing and bone regeneration in mandibular third molar extraction sockets. The study was conducted in 20 patients requiring extraction of bilateral mandibular third molar, following extraction. Platelet rich fibrin (PRF) was placed in one extraction sockets, the other socket was studied as the control sites with no PRF. The patient were assessed for post operative pain, soft tissue healing and trabecular pattern in healing bone. Results showed that the evaluation of bone density by radiological assessment showed the grey level value which was calculated after 3 months at the PRF site well comparatively higher than the average baseline value of the bone density at the extraction site in control site. They concluded that autologous PRF was biocompatible and had significantly improved soft tissue healing.

Pradeep et al. (2012)[38] compared and evaluated the effect of autologous Platelet rich fibrin and Platelet rich plasma in the treatment of 3 wall intrabony defects. 90 intrabony defects in 56 subjects were treated either with autologous platelet rich fibrin and open flap debridement or autologous platelet rich plasma and open flap debridement or open flap debridement alone. Clinical parameters such as probing depth, clinical attachment level and radiologic evaluation of the distance from the crest of the bone to the base of the defect were recorded at baseline and 9 months post operatively. All clinical and radiological parameters were improved in sites treated with autologous Platelet rich fibrin and it was concluded that Platelet rich fibrin preparation is less time consuming and less technique sensitive and therefore a better option than Platelet rich plasma.

Pradeep et al. (2012)[39] evaluated the effect of a combination of Porous hydroxyapatite graft with Platelet rich fibrin for the treatment of 3 wall intrabony defects. 90 intrabony defects in 62 subjects were treated with autologous platelet rich fibrin with open flap debridement or platelet rich fibrin plus hydroxyapatite graft with open flap debridement or open flap debridement alone. Clinical and radiographic parameters such as probing pocket depth, clinical attachment level, intrabony defect depth and percentage of defect fill were measured at baseline and 9 months postoperatively. The results of the study revealed that, treatment with Platelet rich fibrin plus open flap debridement and platelet rich fibrin plus hydroxyapatite with open flap debridement provided significant improvements in probing depth, clinical attachment level and radiographic bone fill compared to open flap debridement alone. It was concluded that the addition of hydroxyapatite with Platelet rich fibrin was found to increase the regenerative effect observed with Platelet rich fibrin alone.

Vijayalakshmi et al (2012)[40] presented a case report of 30 year old female in 21 region where the fenestration defect around an implant was treated by the application of platelet rich fibrin, along with bone graft, and guided tissue regeneration membrane. Six months after the GBR treatment, intra oral examination with the bone meter revealed adequate buccolingual width of the ridge of 7 mm. They concluded that a fenestration defect was effectively treated by the application of growth factors both to the bone graft and GTR membrane.

Lekovic et al (2012)[41] examined the suitability of autologous PRF as regenerative treatment for periodontal intrabony defects in humans and to examine the ability of Bovine porous bone mineral (BPBM) to augment the regenerative effects exerted by PRF. 17 paired intrabony defects were randomly treated either with PRF or with PRF–BPBM combination in a split mouth design. Results indicated that PRF can improve clinical parameters associated with human intrabony periodontal defects, and BPBM has the ability to augment the effects of PRF in reducing pocket depth, improving clinical attachment levels and promoting defect fill.

Hitesh Megharaj Desarda et al (2013)[42] evaluated the effectiveness of platelet rich fibrin (PRF) in periodontal regeneration in two patients diagnosed with generalized aggressive periodontitis (GAgP) patients. Clinical and radiographic examination was performed at baseline and 9 months post operatively which showed decreased probing pocket depth, increased attachment level and radiographic bone fill when baseline and 9 month follow up data was compared.

Baiju RM et al (2013)[43] investigated the clinical and radiographic results of a Grade II mandibular furcation defect treated with PRF. 36 year old female with probing pocket depth of 7mm, periodontal attachment loss of 8 mm and grade II furcation involvement was treated with PRF along with alloplastic bone graft. Re-examination after 6 months revealed reduction of PPD, PAL with no sign of bleeding on probing & significant bone formation. Hence, PRF has been shown to be an effective regenerative material in the

management of Grade II furcation, displaying greater reduction in pocket depths and gain in clinical attachments with significant radiographic evidence of bone fill.

Bajaj et al. (2013)[44] compared the additional efficacy of autologous Platelet rich fibrin (PRF) and Platelet rich plasma (PRP) with open flap debridement (OFD) in the treatment of mandibular degree II furcation defects. 72 buccal mandibular degree II furcation defects in 42 patients were treated with either autologous platelet rich fibrin with open flap debridement or autologous platelet rich plasma with open flap debridement or open flap debridement alone. Clinical parameters such as probing depth, relative vertical and horizontal clinical attachment level and radiographic evaluation of bony defect were recorded at baseline and 9 month postoperatively. The results of the study revealed that the sites treated with platelet rich fibrin and Platelet rich plasma showed significant improvement in all clinical parameters and radiographic parameters compared to OFD alone. They concluded that autologous rich fibrin and Platelet rich plasma are two regenerative materials that can be used in the treatment of furcation defects.

Bansal et al. (2013)[45] evaluated the efficacy of demineralized freeze dried bone allograft (DFDBA) combined with Platelet rich fibrin in the treatment of periodontal intrabony defects. 10 patients with bilateral identical intrabony defects were treated with demineralized freeze dried bone allograft alone or demineralized freeze dried bone allograft combined with Platelet rich fibrin. Clinical and radiographic parameters were recorded at baseline and at 6 months post operatively. The results revealed significant radiographic defect fill and defect resolution for both treatment groups and combination

therapy provided better results in terms of probing pocket depth reduction, gain in clinical attachment level compared to treatment with demineralized freeze dried bone allograft alone, suggesting the regenerative potential of platelet rich fibrin in periodontal wound healing.

Qi Li et al (2013)[46] determined the suitability of platelet-rich fibrin (PRF) as a complex scaffold for periodontal tissue regeneration. PRF enhances osteogenic lineage differentiation of alveolar bone progenitors more than of periodontal progenitors by augmenting osteoblast differentiation, RUNX2 expression, and mineralized nodule formation via its principal component fibrin. They also document that PRF functions as a complex regenerative scaffold promoting both tissue-specific alveolar bone augmentation and surrounding periodontal soft tissue regeneration via progenitor-specific mechanisms.

Malathi et al (2013)[47] presented a case report for the management of an intrabony osseous defect with combination of Platelet Rich Fibrin & Bovine derived demineralized bone matrix with clinical and radiographic evaluations for a 6 months follow up period. 40 year old female patient with probing pocket depth (PPD) of 10mm and clinical attachment level (CAL) of 11mm distal to #13. Results revealed there was significant reduction in PPD and CAL gain and radiographic bone fill at the end of 6 months. It was concluded that the combination therapy using platelet rich fibrin with demineralized bone matrix poses to be a rapid, effective and promising grafting modality for the management of intrabony osseous defects.

Shruthi et al (2013)[48] presented a case report of 22 year old male patient with bilateral intrabony and furcation defects. They evaluated the effectiveness of PRF as a regenerative material in comparison with bioactive glass, an alloplast with proven osteogenic potential. Patient received bioactive glass alloplast on one side and autologous PRF on the other side. The 6 months follow up results revealed a significant improvement in clinical parameters and bone fill in both the sites.

Girish Rao et al (2013)[49] evaluated the effects of autologous platelet rich fibrin gel (PRF gel) on bone regeneration following extraction. 22 patients requiring bilateral transalveolar third molar extractions were included and one side was randomly chosen as case and the other side was taken as control. Autologous platelet rich fibrin gel was prepared from fresh blood obtained from the patient. The PRF gel was placed in the extraction site and primary closure was obtained. Results revealed that the amount of radiographic bone filling was greater in PRF placed site.

Mirjana Gojkov-Vukelic et al (2013)[50] estimated the efficiency of application of diode lasers in the reduction of periodontal pockets in 24 subjects. All subjects underwent general anamnesis, periodontal status, and orthopantogram radiograph analysis and microbial estimation of subgingival plaque prior to laser irradiation of periodontal pockets, immediately following irradiation, and during the control examination 3 months after irradiation was done. The results showed that there was a statistically significant decrease in CT values for the tested bacteria immediately after treatment and the control examination, compared with the level of CT values for the same bacteria before

treatment. Based on the obtained results, they concluded that diode laser irradiation reduces the number of active periodontal pathogens.

Rosamma Joseph et al (2014)[51] evaluated the clinical effectiveness of autologous Platelet Rich Fibrin (PRF) in the treatment of horizontal bony defects. A total of 45 sites with horizontal bone loss in 15 patients were studied, 15 sites were treated with PRF gel and 15 sites were treated with PRF gel and PRF membrane. Control group (15 sites) were treated with open flap debridement. Results revealed that all groups showed a significant reduction in probing depth and clinical attachment gain as compared to baseline. There was no significant difference in gingival recession and radiographic bone levels at 9 months post operatively in all the three groups. They concluded that, clinically the use of PRF in both gel and membrane form was more effective than open flap debridement alone in the management of horizontal periodontal defects at nine months post operatively.

Lakshmi et al. (2014)[52] evaluated the effectiveness of a combination of Platelet rich fibrin and bioactive glass, Platelet rich plasma and bioactive glass and bioactive glass alone in the treatment of 30 intrabony defects in 17 patients. Clinical parameters including probing depth, clinical attachment level and marginal recession were measured at baseline, 3, 6 and 9 months post operatively. The results revealed that the treatment of intrabony defects with test materials showed significant improvements in all the parameters compared to baseline measurements. It was concluded that Platelet rich fibrin

displays slightly superior effect compared to Platelet rich plasma, which in turn displayed superior efficacy than bioactive glass alone.

Mishal P. Shah et al (2014)[53] evaluated the effectiveness of platelet rich fibrin for the treatment of intrabony defect associated with labial-cervical-vertical groove. 47 year old patient with probing pocket depth of 11mm in 21 with no mobility & tear shaped radiolucency was present with localized bone loss in 21. Clinical examination revealed labial cervical vertical groove in 21. Labial cervical vertical groove was sealed with glass ionomer cement and associated intrabony defect was treated with PRF. Clinical and radiographic examination was done at 3 months and 6 months post operatively. The probing pocket depth was reduced to 2mm and bone regeneration was noticed in radiograph. They concluded that periodontal condition was stable and bone regeneration was evident at grafted site.

George et al (2014)[54] evaluated an approach combining platelet rich fibrin for the treatment of osseous defect and a modified crown preparation technique to reposition the crown was done on a pathologically migrated right lateral incisor in a 18 year old female. Deep periodontal pocket was present distally and the tooth showed Grade II mobility. Intraoral periapical radiograph showed bone loss distally till apical third of the root along with periapical radiolucency. Scaling and root planing and root canal treatment was done which was followed by periodontal surgery with PRF placement. After 8 weeks the probing pocket depth had reduced to 3 mm in the distal site, but there was no spontaneous closure of the anterior space which was present before. A modified crown

preparation was done on affected tooth such that the resulting crown would close the anterior space in addition to provide a long term support to root canal treated tooth. Six months following treatment, the patient presented with a clinically healthy gingiva and a probing pocket depth of 3 mm and significant bone fill in radiograph. They concluded that interdisciplinary management including periodontal treatment using PRF, endodontic and restorative therapy may be an effective method in restoring the periodontal health and esthetics of a pathologically migrated tooth.

Parupalli Karunakar et al (2014)[55] evaluated two case reports with primary periodontal lesion with secondary endodontic involvement. In both cases root canal treatment was done which is followed by periodontal surgery along with PRF placement. Post operatively, 9 months showed absence of an intraradicular lesion, pain and swelling, along with tooth stability and adequate radiographic bone fill.

Davoud Zare et al (2014)[56] evaluated effect of diode laser (980 nm) on gingival inflammation when it is used between the first and second phase of periodontal treatment and in comparison with common treatment (SRP) modality alone. 21 patients with moderate to severe chronic periodontitis were selected and divided into control group (Scaling and root planing) and test group (Scaling and root planing + laser). Two months after the last scaling and laser radiation, indices including gingival level (GL), bleeding on probing (BOP) and modified gingival index (MGI) were recorded and compared with baseline. Results revealed that all indices improved in both groups except for BOP which was lower in laser group. They concluded that based on overall improvement in

parameters like superiority of laser application in some indices, lack of thermal damage and gingival recession with the specific settings used, the application of laser as an adjunctive treatment together with common methods is preferable.

Mahitab Mahmoud M. Solimanet al (2014)[57] carried out research to assess the therapeutic effects of Diode Laser (DL) on chronic periodontitis, by reducing pockets depth and minimizing Microbial Counts (MCs). 50 patients with chronic periodontitis were divided into two groups. All patients were subjected to scaling, one group received DL therapy and the other group received same treatment but instead of DL therapy irrigation with normal saline. The operation period (10weeks) were divided into: phase1 (baseline) at 1^{st} week, phase2 (treatment sessions) at 2^{nd} , 4^{th} , and 6^{th} week, and phase3 (follow up) at 10^{th} week. Clinical parameters evaluation and MCs were detected during the operation period. Index of Bleeding on Probing (BOP) had been improved greatly in group treated with diode laser as 96.9%, while other group as 20.5%. Plaque Index (PI), and Pocket Depths (PD) and Colony Forming Units/ml were more reduced in diode laser group than other group.

Lata Goyal (2014)[58] evaluated the efficacy of PRF and alloplastic bone substitute in the management of intrabony defect associated with endo-perio lesion. A 35 year old patient with endo perio lesion in maxillary lateral incisor with probing pocket depth of 8mm. Scaling and root planing and endodontic treatment was done which was followed by flap surgery with PRF placement along with bioactive glass. It was observed that at 3, 6, 9 and 12 months follow-up after the surgical treatment of large chronic periapical lesion,

PRF combined with β-TCP resulted in significant clinical and radiographic bone regeneration.

A.R. Pradeep et al (2015)[59] evaluated the efficacy of open-flap debridement (OFD) combined with PRF, 1% Metformin gel, and PRF + 1% MF gel in the treatment of intrabony defects (IBDs) in patients with chronic periodontitis (CP). One hundred twenty patients with single defects were categorized into four treatment groups: OFD alone, OFD with PRF, OFD with 1% MF, and OFD with PRF plus 1% MF. Clinical parameters such as site-specific plaque index (PI), modified sulcus bleeding index (mSBI), probing depth (PD), relative attachment level (RAL), and gingival marginal level (GML) were recorded at baseline (before surgery) and 9 months postoperatively. Results showed that PRF, 1% MF, and PRF + 1% MF groups showed significantly more PD reduction and RAL gain than the OFD only group. Mean PD reduction and mean RAL gain were found to be greater in the PRF + 1% MF group compared to just PRF or MF at 9 months. Furthermore, PRF + 1% MF group sites showed a significantly greater percentage of radiographic defect depth reduction compared to MF, PRF, and OFD alone at 9 months. They concluded that The PRF + 1% MF group showed greater improvements in clinical parameters, with greater percentage radiographic defect depth reduction compared to MF, PRF, or OFD alone in treatment of IBDs in patients with CP.

Shah et al. (2015)[60] investigated the effect of Platelet rich fibrin in the regeneration of periodontal intrabony defects and compared it with demineralized freeze dried bone allograft in 20 patients with bilateral defects. The intrabony defects received two plugs of

PRF prepared from patients own blood or demineralized freeze dried bone allograft. Clinical parameters such as probing depth, relative attachment level and gingival margin level were measured at baseline and 6 months post surgery. The results of the study revealed that Platelet rich fibrin showed significant improvement after 6 months which was comparable to demineralized freeze dried bone allograft for periodontal regeneration. They concluded that Platelet rich fibrin can be used in the regeneration of periodontal intrabony defects.

Ajwani et al. (2015)[61] evaluated the clinical efficacy of platelet rich fibrin and open flap debridement in the treatment of intrabony defects. 20 subjects with forty intrabony defects were treated either with platelet rich fibrin with open flap debridement or with open flap debridement alone. Clinical parameters recorded at baseline and at 9 months postoperatively included plaque index, sulcus bleeding index and relative attachment level. The results revealed that a statistically significant improvements were seen in the sites treated with Platelet rich fibrin compared to control sites. Similarly the adjunctive use of platelet rich fibrin with open flap debridement significantly improved the radiographic defect fill.

Elgendy et al. (2015)[62] investigated the effect of nanocrystalline hydroxyapatite bone graft with or without Platelet rich fibrin membrane in the treatment of intrabony periodontal defects in 20 patients with bilateral identical intrabony defects. Clinical parameters such as probing depth, clinical attachment level and radiographic evaluation of the defect were recorded at baseline and at 6 months post operatively. The results of

the study revealed that nanocrystalline hydroxyapatite bone graft in combination with platelet rich fibrin demonstrated significant probing pocket depth reduction, clinical attachment level gain and increased radiographic bone density than nanocrystalline hydroxyapatite alone.

Nagaveni et al (2015)[63] described the efficacy of PRF in the treatment of intra bony defect associated with an endoperio lesion in an immature right mandibular first premolar of 12-year-old female patient. The presence of deep periodontal pocket measuring 13 mm mesially was observed and radiograph showed deep intrabony defect on the mesial aspect of the tooth extending to the apical region of the root. The tooth had an immature root, thin dentinal walls with wide open apex. Flap surgery is done and PRF membrane was placed into the bony defect to the level of surrounding bony walls, taking care not to overfill. The periodontal probing pocket depth was found to reduce to 2 mm 6 months post surgically. Radiographic examination showed a significant bony fill in the defect. At 6-month follow-up, the radiograph showed complete bone fill similar to adjacent normal teeth.

Vineetha Varughese et al (2015)[64] evaluated the combination therapy of a blend of platelet rich fibrin with bone graft and guided tissue regeneration membrane was used in the treatment of a perioendo lesion of a multirooted tooth. Periodontal examination at six months and 12 months revealed a reduction in probing pocket depth, normal soft tissue contour and no mobility. They concluded that the combination therapy of a blend of PRF

and bone graft and GTR showed successful results with elimination of pain, mobility and also improvement in periodontal parameters and patient satisfaction.

Meenu Dhiman et al (2015)[65] evaluated the healing outcomes of platelet-rich fibrin (PRF) in periapical surgeries with apicomarginal defects and to compare these results with surgeries not using any guided tissue regeneration techniques. Thirty patients with suppurative chronic apical periodontitis with apicomarginal communication were randomly assigned to either the PRF or the control group. Results revealed that both the groups exhibited a significant reduction in Probing depth, clinical attachment level, gingival marginal position, and size of periapical lesion at 12-month. They concluded that the adjunctive use of regenerative techniques may not promote healing of apicomarginal defects of endodontic origin. No significant differences were observed between the two groups for these parameters except PD, which showed a statistically significant reduction in the PRF group.

Gabriela Beresescu et al (2015)[66] assess the histological efficiency of low level laser therapy (LLLT) with respect to the acceleration of bone regeneration after surgical treatment of intrabony defects. Twenty patients with intrabony defects were selected among which 10 received low level laser therapy by histological analysis. Results revealed that test samples at 6 months after regeneration showed bone formation without inflammatory cells.

Tanya et al (2015)[67] aimed to investigate the adjunctive effect of diode laser irradiation with open flap debridement (OFD), while treating chronic periodontitis. A total of 30

patients with generalized chronic moderate to severe periodontitis with pocket probing depth (PD) ≥5 mm were selected for a split mouth study. Flap surgery with adjunctive diode laser irradiation was performed in the test quadrant while routine OFD was done in the control quadrant. Clinical parameters including PD, clinical attachment level, gingival recession, plaque index, gingival index and tooth mobility were recorded at baseline, 3 months and 6 months following treatment. Results showed that all clinical parameters significantly improved after therapy without any statistically significant difference between the two groups for any of the parameters and the exception was that there is a significantly greater reduction in gingival inflammation in the laser treated group. It was concluded that the diode laser can be safely and effectively used as an adjunct during the treatment of chronic periodontitis with the advantage of decreased gingival inflammation.

Sam et al (2015)[68] evaluated the mechanical properties of the platelet rich fibrin (PRF) membrane and compared these properties with that of commercially available collagen membranes used for guided tissue regeneration (GTR) procedures. Modulus of elasticity, hardness and invitro degradation of PRF membrane, bovine collagen membrane and fish collagen membrane were assessed. They concluded that the preliminary findings from the assessment of the mechanical properties of PRF membrane showed it was lacking in several desired properties when compared to commercially available collagen membranes. Lack of rigidity and faster degradation may limit its application in GTR procedures.

Aydemir Turkal et al (2016)[69] compared the results obtained with enamel matrix derivative (EMD) and EMD + platelet-rich fibrin (PRF) in the treatment of intrabony defects (IBDs) in chronic periodontitis patients. 28 paired IBDs were randomly treated either with EMD or with EMD + PRF in a split mouth design. Clinical and radiographic measurements including clinical attachment level, probing depth (PD), gingival recession (GR), defect depth (DD), defect width (DW) and defect angle (DA) were recorded at baseline (BL) and at six months following therapy. Results revealed that BL clinical and radiographic measurements were similar for EMD and EMD + PRF groups. Although postsurgical measurements revealed significant reduction for PD and CAL in both groups, no intergroup difference was detected and defect fill was not also statistically different. They concluded that both therapies resulted in significant clinical improvement in IBD treatment and PRF did not improve the clinical and radiographic outcomes.

Akhilesh Tomar et al (2016)[70] evaluated the effects of platelet rich fibrin (PRF) on growth and proliferation of cultured human dental pulp stem cells, PRF induced alterations in the expression of senescence and apoptosis markers and influence of platelet rich fibrin (PRF) on osteogenic differentiation potential of human dental pulp stem cells (hDPSCs). Results showed that growth factors released from PRF can support hDPSCs proliferation and differentiation and also showed significant higher activity of osteogenic markers like Alp, Osn, Osp, Osc and Runx2.

Santosh Dixit et al (2016)[71] evaluated the effect of a diode laser with nonsurgical periodontal therapy on chronic periodontitis. A 37year old female, with chronic

periodontitis was treated with 940 nm diode laser and scaling and root planning. Following laser therapy assessment of clinical parameters was done after 6 months; the probing depths improved; gain in clinical attachment levels; no inflammation; the tissue tone was good, showing increased stippling.

Santosh S. Martande et al (2016)[72] evaluated the combined efficacy of PRF and 1.2% Atorvastatin (ATV) gel with open flap debridement (OFD) in treatment of intrabony defects in chronic periodontitis (CP) individuals. Ninety six individuals with single defects were categorized into three groups: OFD with PRF, OFD with PRF+1.2% ATV and OFD alone. Clinical parameters like site specific plaque index (PI), modified sulcus bleeding index (mSBI), probing depth (PD), relative attachment level (RAL) and gingival marginal level (GML) were recorded at baseline before surgery and 9th month post-operatively. Percentage of radiographic intra-bony defect depth reduction was evaluated at baseline and 9 months. Clinical parameters were improved similarly in all the groups but the amount of bone fill was greater in PRF alone group and hence it was concluded that 1.2% ATV failed to augment the regenerative potential of PRF alone in periodontal intrabony defects.

A R Pradeep et al (2016)[73] assessed the combined effectiveness of PRF and 1% Alendronate (ALN) with access therapy in treating intrabony defects in patients with chronic periodontitis. Single intrabony defects in ninety patients were categorized into: Group 1(Access therapy alone), Group 2 (Access therapy with PRF), and Group 3 (Access therapy with PRF+1% ALN). Site specific plaque index, modified sulcus bleeding index (mSBI), probing depth (PD), clinical attachment level (CAL) and gingival marginal level (GML) included as parameters for clinical assessment, were evaluated

before surgery at baseline and post-operatively (9 months). It was concluded that combined approach therapy of PRF+1% ALN for intrabony defects treatment in CP patients showed better clinical parameters outcomes with greater defect depth reduction in comparison to PRF and access therapy alone.

A R Pradeep et al (2016)[74] evaluated and compared the efficacy of open-flap debridement (OFD) with or without PRF or PRF + 1.2% Rosuvastatin (RSV) gel in the treatment of intrabony defects (IBDs) in chronic periodontitis (CP) patients. Ninety individuals with a total of 90 IBDs were randomly assigned to one of the 3 treatment groups: 1) OFD alone, 2) OFD + PRF and 3) OFD + PRF + 1.2% RSV gel placement. Plaque index (PI),modified sulcus bleeding index (mSBI), probing depth (PD), clinical attachment (CA) level and IBD depth were recorded at baseline and at 9 months post-operatively. It was concluded that 1.2% RSV with PRF results in significantly greater periodontal benefits compared to OFD alone or with PRF.

Materials and Methods

MATERIALS AND METHODS

PATIENT SELECTION:

The patients who participated in the study were selected from out patients who visited the Department of Periodontology, Best Dental Science college & hospital, Madurai. A total of 10 patients including both males and females aged between 20- 60 years were selected. The study subjects were clinically (using William's probe) and radiographically evaluated for the presence of intrabony defect.

The study protocol was approved by the Institutional Ethical Committee and Review Board (18.11.2014). Written and verbal consent was obtained from the selected patients. All the patients included in the study satisfied the following inclusion and exclusion criteria.

INCLUSION CRITERIA:

1. Age between 20 and 60 years.
2. Patients with good oral health and without any history of systemic disease.
3. Generalized chronic periodontitis with probing pocket depth (PPD) of $\geq$ 5 mm and presence of intrabony defects on contralateral sides of same arch or in opposite arches in an intraoral periapical radiograph.

EXCLUSION CRITERA:

1. Patients with known systemic illness like diabetes or other systemic illness.
2. Taking any medications known to affect the outcomes of periodontal therapy.
3. Pregnancy or lactation.
4. Patients using any form of tobacco were excluded from the study.

RANDOMIZATION

Randomization of the defect site was selected by tossing a coin. Experimental site B were treated with laser disinfection and platelet rich fibrin placement and Experimental site A were treated with open flap debridement and platelet rich fibrin.

ARMAMENTARIUM:

ARMAMENTARIUM FOR CLINICAL EVALUATION:

1. Mouth mirror.

2. Explorer.

3. William's calibrated periodontal probe.

4. Customized occlusal stents.

ARMAMENTARIUM FOR RADIOGRAPHIC EVALUATION

1. Intra - oral periapical radiograph (3 E Speed).

2. XCP holder.

3. X - ray Digitizer.

SURGICAL ARMAMAMENTARIUM:

1. Sterile surgical gloves.
2. Mouth mirror.
3. William's calibrated periodontal probe.
4. Explorer.
5. Cotton pliers.
6. Sterilized cotton pellets and gauze.
7. Povidone iodine.
8. Bard Parker handle no. 3.

9. Surgical blade no. 15
10. Periosteal elevators.
11. Straight scissors.
12. Castroviejo scissors.
13. Gracey curettes.
14. Needle holder.
15. Non resorbable 3-0 black braided silk suture.
16. Diode laser.
17. 2% lignocaine local anesthetic agent containing adrenaline in the ratio of 1:80,000.
18. Normal saline.

ARMAMENTARIUM FOR PRF PREPARATION

1. PRF box.
2. Sterile test tube.
3. Sterile cotton pliers.
4. Sterile scissors.
5. Sterile surgical gloves.
6. 10ml syringe.
7. Tourniquet.
8. Table centrifuge.

CLINICAL PARAMETERS

1. Plaque Index (Modification by Loe 1967)[75]

2. Gingival Index (Modification by Loe 1967) [75]

3. Probing pocket depth[76]

4. Clinical attachment level. [76]

PLAQUE INDEX:

Plaque index was described by **Silness and Loe in 1964 and modified by Loe in 1967**.

The scores and the interpretation of the plaque index are given below.

Score	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen only by running a probe across the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen by the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface

Calculation of plaque index:

Plaque index for the area: Each area (disto-facial, mesio-facial, facial and lingual) is assigned a score from 0-3

Plaque index for a tooth: The scores from the four areas are calculated and divided by four.

Plaque index score for the individual: The scores for each tooth were added and then divided by the total number of teeth examined.

INTERPRETATION:

Excellent	0
Good	0.1-0.9
Fair	1.0-1.9
Poor	2.0-3.0

GINGIVAL INDEX:

The Gingival Index (GI) was developed by **Loe and Silness in 1963 and modified by Loe in 1967.**

Instrument used: Mouth mirror and periodontal probe.

The tissues surrounding each tooth are divided into four gingival scoring units: distofacial papilla, facial margin, mesio-facial papilla and the entire lingual gingival margin. The teeth and gingiva should be dried lightly with a blast of air and /or cotton rolls.

Each of the 4 gingival units was assessed according to the criteria as follows:

Score	**Criteria**
0	Absence of inflammation/normal gingiva.
1	Mild inflammation: slight change in color, slight edema; no bleeding on

	probing
2	Moderate inflammation: moderate glazing, redness, edema and hypertrophy,bleeding on probing.
3	Severe inflammation: marked redness and hypertrophy, ulceration, tendency to spontaneous bleeding.

Calculation:

Gingival index Score for the area: Each area (disto-facial, facial, mesio-facial, lingual) is assigned a score from 0 to 3.

Gingival index Score for a tooth: The scores from the four areas of the tooth are added and then divided by four.

Gingival index score for the individual: The indices for each of the teeth are added and then divided by the total number of teeth examined. The scores range from 0 to 3.

INTERPRETATION:

Gingival scores	Condition
0.1-1.0	Mild Gingivitis
1.1-2.0	Moderate Gingivitis
2.1-3.0	Severe Gingivitis

Probing pocket depth was measured using a Williams'calibrated periodontal probe in the mid buccal or mid lingual area with the fabricated stent in place.

Clinical attachment level:

Clinical attachment level is the distance between the base of the pocket and a fixed point on the crown, such as the cementoenamel junction.

When the gingival margin is located on the anatomic crown the level of attachment is determined by subtracting from the depth of the pocket the distance from the gingival margin to the CEJ. If both are same, the loss of attachment is zero.

When the gingival margin coincides with the CEJ, the loss of attachment equals the pocket depth.

When the gingival margin is located apical to the CEJ, the loss of attachment is greater than the pocket depth and therefore the distance between the CEJ and the gingival margin is added to the pocket depth.

RADIOGRAPHIC PROCEDURE

Intra-oral periapical radiographs were taken using long cone paralleling angle technique. Study subjects were made to wear lead apron and thyroid collar before x – ray exposure. Subjects were then positioned upright in the chair with proper back support. The x ray unit settings for the Kvp, mA and tube head angulations were adjusted according to the region of interest. X rays taken were developed and digitalized. Radiographic assessment was done by using Image J analysis software.

RADIOGRAPHIC PARAMETER

Intra oral periapical radiographs were taken at baseline and 6 months after treatment.

PLATELET RICH FIBRIN PREPARATION PROTOCOL:

Platelet rich fibrin was prepared according to the protocol developed by **Choukroun et al in the year 2001**. Prior to the surgery 10ml of intravenous blood was collected from the

patient's antecubital vein and transferred into a sterile glass test tube without any anticoagulant. The test tube was then placed in the centrifuge machine immediately and centrifuged for 10 minutes at 3000rpm. Blood centrifugation resulted in the separation of the blood components into three layers. Platelet poor plasma is collected in the topmost layer and the bottom layer is the RBC base. The layer between these two is the fibrin clot into which all the platelets were trapped. PRF clot was retrieved from the test tube using sterile tweezers and then separated from the underlying RBC base using a sterile scissors preserving some amount of RBC.

PRE SURGICAL THERAPY

Surgical intervention were carried out after complete scaling and root planing with proper oral hygiene instructions. After phase I therapy the patients were kept in maintenance phase for a period of 6-8 weeks and re-evaluated to confirm the suitability of the sites for the surgery. The test site and the control sites were clinically and radiographically evaluated at baseline and 6 months post operatively.

SURGICAL PROTOCOL

Pre-surgical oral rinse was advised prior to the surgery. Intraoral and extraoral antisepsis was maintained using povidone iodine. After administrating local anesthesia (2% lignocaine in the ratio of 1:80,000) and achieving proper anesthesia, intrasulcular and interdental incisions were given using 15 size BP blade. Full thickness mucoperiosteal flap were elevated and root debridement and curettage was done using area specific Gracey curettes. Intrabony defect was identified. The surgical area was irrigated with sterile normal saline. Pre-suturing was done using non resorbable 3-0 black braided silk. Experimental site B received platelet rich fibrin and laser disinfection. The

prepared PRF was gently packed into the intrabony defect and overfilling was avoided. Experimental site A also received the identical treatment except for the laser disinfection.

POST OPERATIVE CARE

Suture were removed one week after surgery. The operated area was rinsed carefully with normal saline. Gentle brushing with a soft brush was recommended.

Oral hygiene maintenance was reinforced in all the patients. The patients were recalled every one week for first month and thereafter 6 months post operatively. All the clinical measurements were repeated at 1, 3 and 6 months post operatively and radiographs were repeated at the end of the 6 months.

FIG.1 ARMAMENTARIUM FOR CLINICAL EVALUATION

FIG.2 ARMAMENTARIUM FOR RADIOGRAPHIC EVALUATION

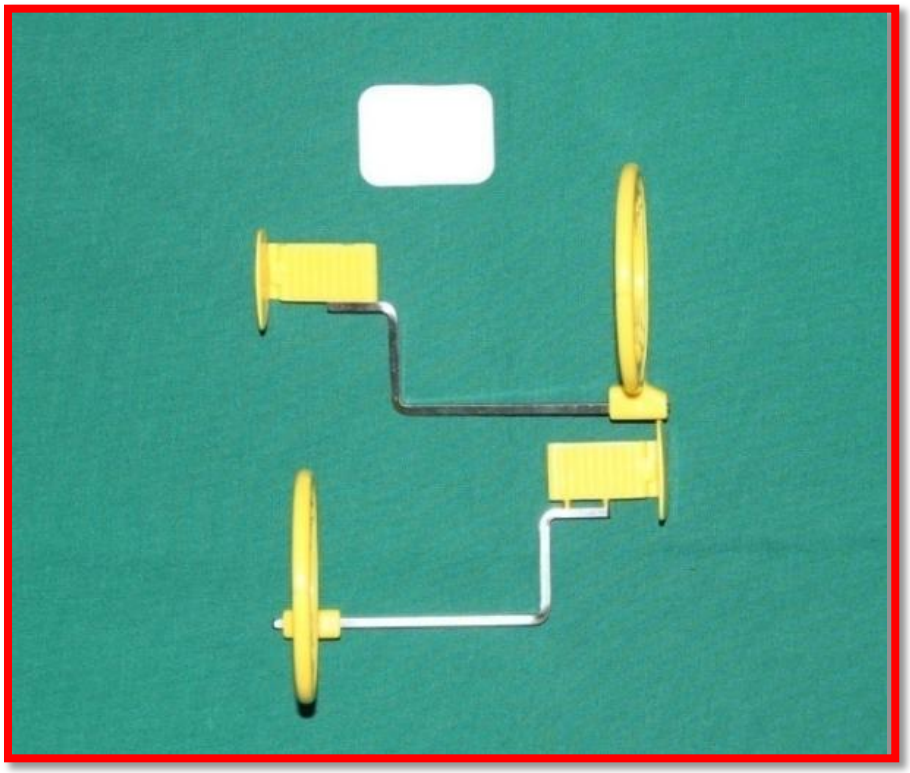


FIG.3 ARMAMENTARIUM FOR PRF PREPARATION

FIG.4 ARMAMENTARIUM FOR SURGICAL PROCEDURE

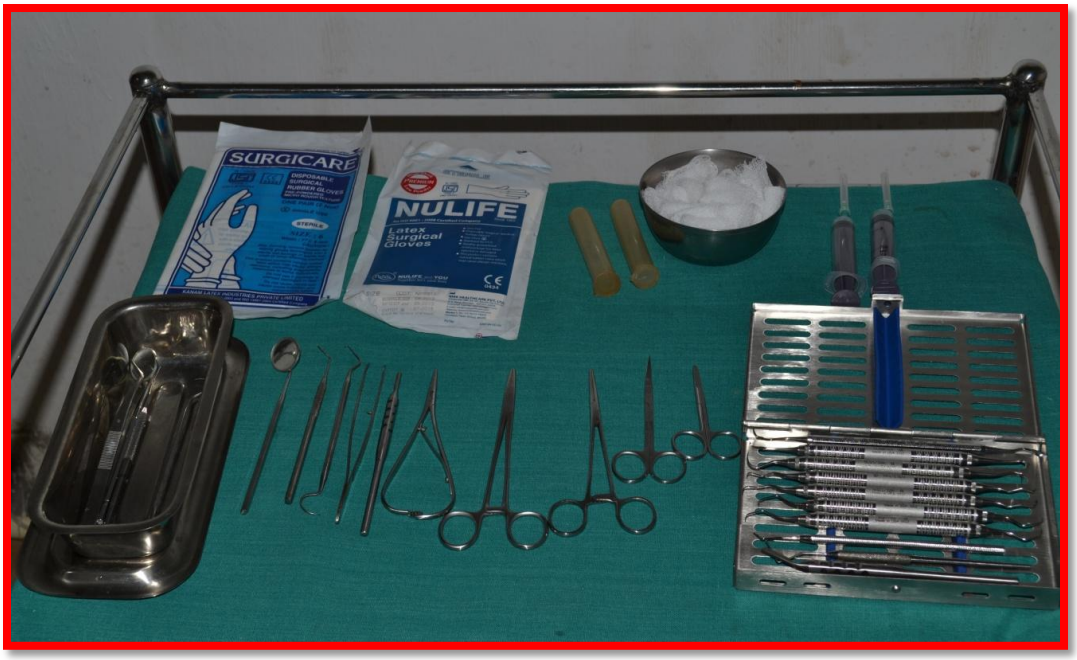


Fig.5 PRF PREPARATION PROCEDURE

Collecting 10ml of blood

Centrifugation

After centrifugation

Platelet Rich Fibrin

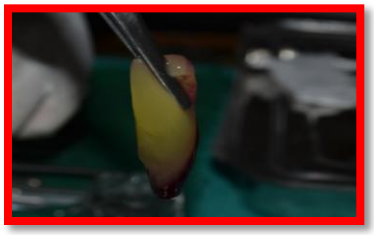
Fig.6 SURGICAL PROCEDURE – EXPERIMENTAL SITE A

Pre operative probing pocket depth

Sulcular Incision given

Full thickness flap elevated and debridement done

Pre – sutures placed



Procurement of Platelet rich fibrin

Placement of PRF into defect site

Sutures placed

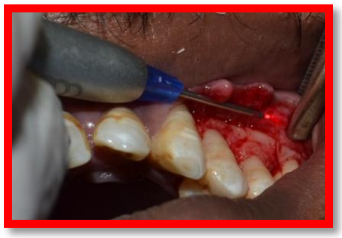
Post operative probing Pocket depth

Fig.7 SURGICAL PROCEDURE – EXPERIMENTAL SITE B

Pre operative probing pocket depth

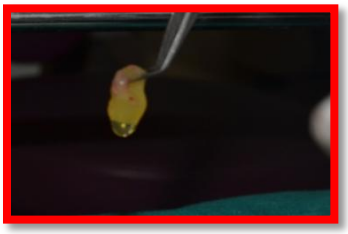
Sulcular Incision given

Full thickness flap elevated and debridement done



Laser debridement

Pre – sutures placed



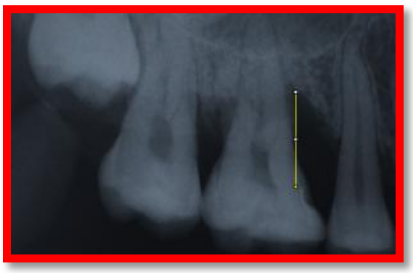
Procurement of Platelet rich fibrin

Placement of PRF into defect site

sutures placed

Post operative probing Pocket depth

Fig.8 PRE AND POST OPERATIVE RADIOGRAPHS



Pre-operative radiograph –

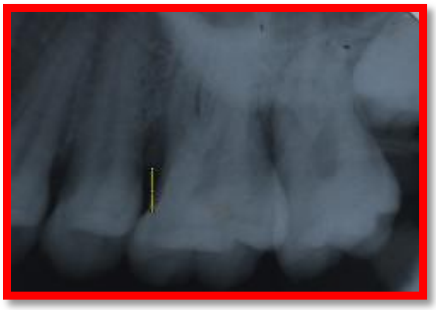
Experimental site A

Post-operative radiograph –

Experimental site A

Pre-operative radiograph –

Experimental site B



Post-operative radiograph –

Experimental site B

Statistical Analysis

STATISTICAL ANALYSIS

All the clinical and radiographic parameters recorded were subjected to the following statistical analysis:

Descriptive analysis that included mean, standard deviation were found for each parameter in two groups and were used for analysis.

1. For intragroup comparison, paired ‘t’ test was performed to compare post treatment changes from baseline.
2. For comparison between the inter-group variations unpaired ‘t’ test was performed.

A ‘p’ value of 0.05 or less was considered for statistical significance.

Results

RESULTS

The present clinical study was conducted to evaluate and compare the efficacy of autogenous platelet rich fibrin assisted with and without laser in the treatment of periodontal intrabony defects and their effect on the clinical and radiographic parameters.

A total of 10 patients satisfying the selection criteria were selected. Twenty sites in these patients showing intrabony defects, were randomly selected according to the split mouth design and divided into experimental site A and experimental site B.

Experimental site A: 10 sites were treated with open flap debridement followed by placement of platelet rich fibrin (PRF).

Experimental site B: 10 sites were treated with laser disinfection (Biolase) followed by placement of platelet rich fibrin (PRF).

The following clinical parameters were recorded at the selected sites at baseline, 1 month, 3 months and 6 months.

- Plaque index
- Gingival index
- Probing pocket depth (PPD) – baseline and 6 months
- Clinical attachment level – baseline and 6 months

Radiographic evaluation:

For the selected sites, intra-oral periapical (I.O.P.A) radiographs were taken at baseline and 6 months using long cone paralleling technique. Mean defect fill was

measured and the radiographic assessment was done by Computer Assisted Image analysis.

Clinical parameters:

Plaque Index: (Table 1&2; Graph 1)

The mean plaque index score at baseline was 1.43 ± 0.09 which was reduced to 0.10 ± 0.05 at 1 month, 0.04 ± 0.02 at 3 months and 0.02 ± 0.02 at 6 months showing a mean reduction of 1.3 ±0.10 at 1 month, 1.38 ± 0.08 at 3 months and 1.4 ± 0.08 at 6 months respectively, which were statistically highly significant with the p value of 0.000 ($p < 0.001$).

Gingival Index: (Table 3&4; Graph 2)

The mean gingival index score at baseline was 1.03 ± 0.30 which was reduced to 0.08 ± 0.06 at 1 month, 0.03 ± 0.02 at 3 months, 0.03 ± 0.02 at 6 months showing a reduction of 0.9 ± 0.2 at 1 month, 1.0 ± 0.3 at 3 months and 1.0 ± 0.3 at 6 months, which were statistically highly significant with the p value of 0.000 ($p < 0.001$).

Probing pocket depth: (Table 5&6; Graph 3&4)

Experimental site A: The mean probing pocket depth at baseline was 8.4 ± 1.5 mm which was reduced to 4.3 ± 0.9 mm at 6 months showing a mean reduction of 4.1 ± 0.9 mm which was highly significant with the p value of 0.000 ($p<0.001$).

Experimental site B: The mean probing pocket depth at baseline was 8.3 ± 2.5 mm which was reduced to 3.6 ± 1.1 mm showing a mean reduction of 4.7 ± 1.6 mm which was highly significant with the p value of 0.000 ($p<0.001$).

Comparison of the mean reduction of probing pocket depth between the two groups at baseline and 6 months were statistically significant with the p value of 0.010.

Clinical attachment level: (Table 7&8; Graph 5&6)

Experimental site A: The mean clinical attachment level at baseline was 8.7 ± 1.9 mm, which was reduced to 5.0 ± 1.5 mm at 6 months, showing a mean gain of 3.7 ± 1.4 mm, which was statistically highly significant with the p value of 0.000 ($p<0.001$).

Experimental site B: The mean clinical attachmrnt level at baseline was 8.8 ± 3.5 mm, which was reduced to 4.5 ± 2.1 mm at 6 months, showing a mean gain of 4.3 ± 1.8 mm, which was statistically highly significant with the p value of 0.000 ($p<0.001$).

Comparison of mean gain in clinical attachment level between the two groups, revealed a difference of 0.5 ± 1.2 mm at 6 months with the p vale of 0.244 which was statistically not significant.

RADIOGRAPHIC EVALUATION:

Mean defect fill: (Table 9&10; Graph 7,8,9,10.)

Experimental site A: The mean distance from CEJ to the base of the defect at baseline was 7.3 ± 2.3 mm which was reduced to 6.4 ± 2.2mm by the 6^{th} month which was statistically highly significant with the p value of 0.000 ($p<0.001$).

Experimental site B: The mean distance from CEJ to the base of the defect at baseline was 6.8 ± 2.9 mm which was reduced to 5.7 ± 2.8 mm by the 6^{th} month which was statistically highly significant with the p value of 0.000 ($p<0.001$).

Comparison between the two groups revealed a difference of 0.72 mm at 6 months was statistically not significant with the p value of 0.542 ($p>0.05$).

Table 1: PLAQUE INDEX

SI NO	BASELINE	1 MONTH	3 MONTHS	6 MONTHS
1	1.5	0.1	0.1	0.09
2	1.4	0.09	0.06	0.03
3	1.4	0.09	0.06	0.03
4	1.5	0.2	0.05	0.04
5	1.4	0.12	0.04	0.02
6	1.2	0.12	0.01	0.01
7	1.5	0.1	0.04	0.04
8	1.5	0.2	0.05	0.009
9	1.4	0.03	0.02	0.01
10	1.5	0.03	0.02	0.02

Table 2: Mean reduction of Plaque Index score at baseline, 1st month, 3rdmonth and 6th month

Time interval	Mean PI ± SD	Difference fom baseline ± SD	Significance (p*)
Baseline	1.4300 ± 0.0949		
1 Month	0.1080 ± 0.0579	1.3220 ± 0.1043	**0.000***
3 Months	0.0450 ± 0.0259	1.3850 ± 0.0857	**0.000***
6 Months	0.0299 ± 0.0241	1.4001 ± 0.0872	**0.000***

*P-Value = < 0.001(highly significant)

Graph 1: Mean Plaque Index scores at baseline, 1st month, 3rd month and 6th months.

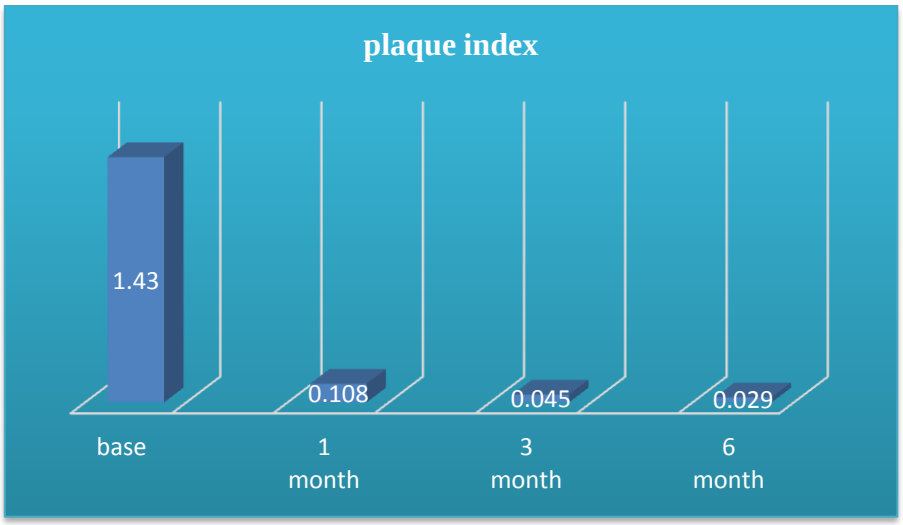


Table 3: GINGIVAL INDEX

SI NO	BASELINE	1 MONTH	3 MONTHS	6 MONTHS
1	0.8	0.1	0.08	0.07
2	1.1	0.2	0.03	0.05
3	1.1	0.2	0.03	0.05
4	1	0.05	0.04	0.02
5	0.85	0.01	0.03	0.04
6	1.01	0.06	0.01	0.01
7	1.3	0.1	0.05	0.03
8	1.5	0.1	0.01	0.02
9	0.4	0.02	0.01	0
10	1.26	0.03	0.01	0.02

Table 4: Mean reduction of Gingival Index scores at baseline, 1st month, 3rd month and 6th month.

Time interval	Mean GI ± SD	Difference from baseline ± SD	P* value
Baseline	1.0320 ± 0.3055		
1 Month	0.0870 ± 0.0682	0.9450 ± 0.2882	**0.000***
3 Months	0.0300 ± 0.0226	1.0020 ± 0.3090	**0.000***
6 Months	0.0310 ± 0.0213	1.0010 ± 0.3046	**0.000***

*P-Value = <0.001(highly significant)

Graph 2: Mean graph for Gingival Index – baseline, 1 month, 3 months and 6 months.

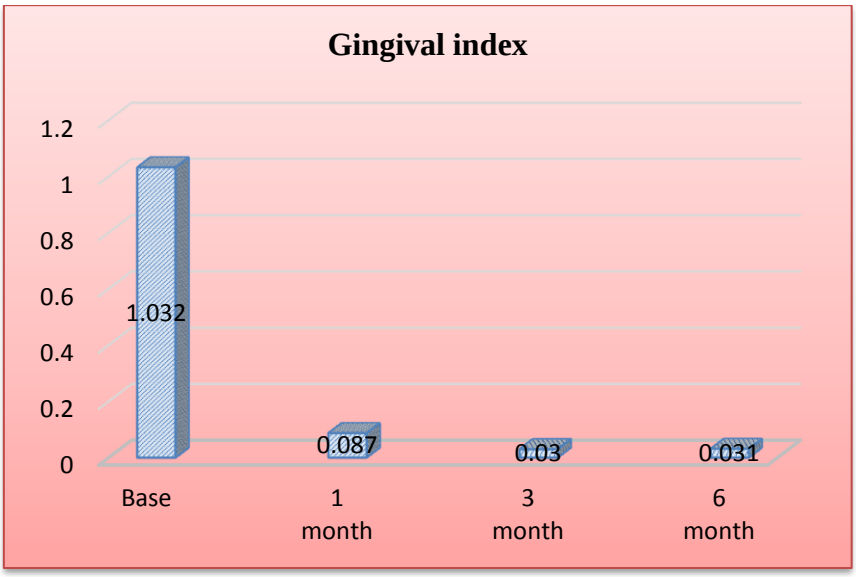


Table 5: PROBING POCKET DEPTH (in mm)

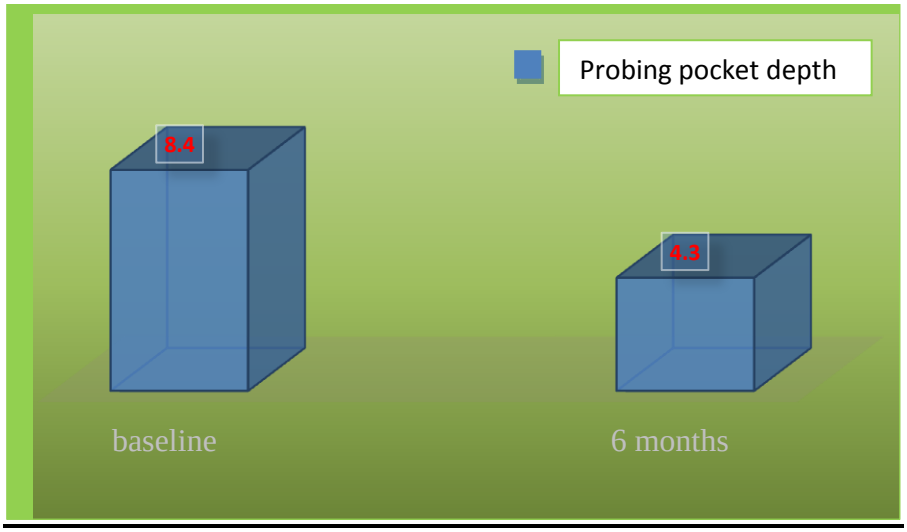
SI NO	Experimental site A		Experimental site B	
	BASELINE	6 MONTHS	BASELINE	6 MONTHS
1	7	4	6	3
2	10	5	8	4
3	10	4	9	3
4	9	5	14	6
5	8	4	6	3
6	6	3	6	2
7	7	4	8	3
8	10	6	10	5
9	7	3	6	3
10	10	5	10	4

Table 6: Mean difference in probing pocket depth of Experimental site A and B (in mm)

Time interval	Experimental site A			Experimental site B			A vs B	
	Mean PPD ± SD (in mm)	Difference from baseline (in mm)	P value	Mean PPD ± SD (in mm)	Difference from baseline (in mm)	P value	Mean difference (in mm)	P value
Baseline	8.400 ± 1.578			8.300 ± 2.584				
6 months	4.300 ± 0.949	4.100 ± 0.994	**0.000 HS**	3.600 ± 1.174	4.700 ± 1.636	**0.000 HS**	0.700 ± 0.675	0.010 S

P-Value = < 0.001 (highly significant). HS-highly significant; S-significant

Graph 3: Comparison of Probing pocket depth between baseline and 6 months (Experimental site A)



Graph 4: Comparison of Probing pocket depth between baseline and 6 months (Experimental site B)

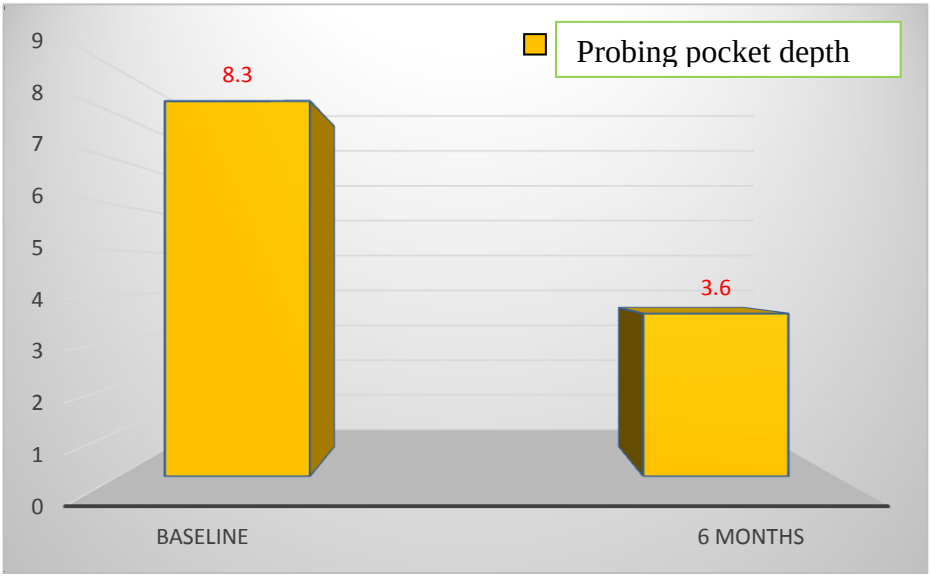


Table 7: Clinical Attachment Level (CAL) In mm

SI NO	Experimental site A		Experimental site B	
	BASELINE	6 MONTHS	BASELINE	6 MONTHS
1	7	5	6	4
2	10	5	8	4
3	10	4	9	3
4	10	6	17	9
5	8	4	6	3
6	6	4	6	3
7	7	4	8	3
8	10	8	10	7
9	7	3	6	3
10	12	7	12	6

Table 8: Mean difference in clinical attachment level of experimental site A and B (in mm)

Time interval	Experimental site A			Experimental site B			A vs B	
	Mean CAL ± SD (in mm)	Difference from baseline (in mm)	p value	Mean CAL ± SD (in mm)	Difference from baseline (in mm)	p value	Mean difference (in mm)	p value
Baseline	8.700 ± 1.947			8.80 ± 3.52				
6 months	5.000 ± 1.563	3.700± 1.418	**0.000 HS**	4.50 ± 2.12	4.300 ± 1.889	**0.000 HS**	0.500 ± 1.269	0.244 NS

p-Value = > 0.05 (not significant).

Graph 5: Comparison of clinical attachment level between baseline and 6 months (Experimental site A)

Graph 6: Comparison of clinical attachment level between baseline and 6 months (Experimental site B)

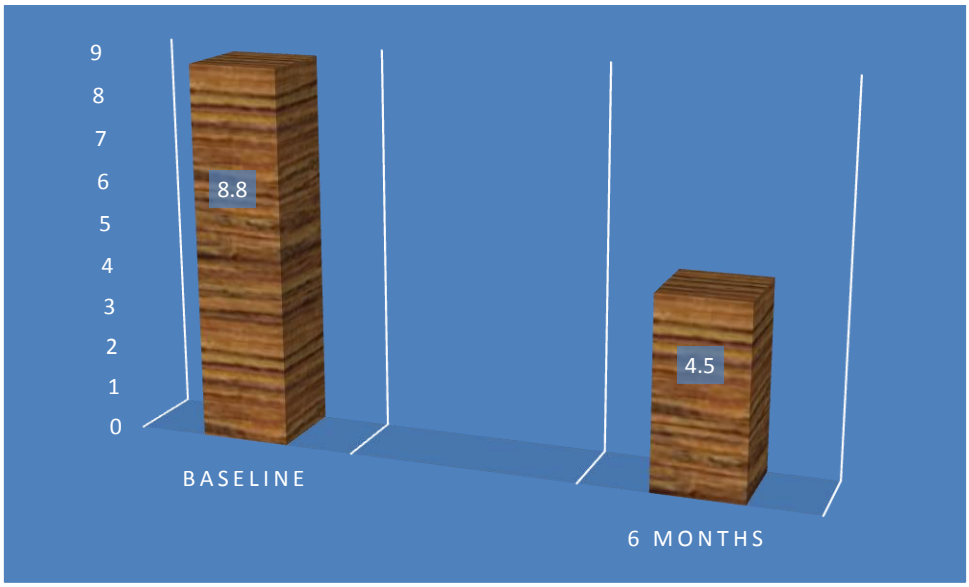


Table 9: CEMENTOENAMEL JUNCTION – BASE OF THE DEFECT (in mm)

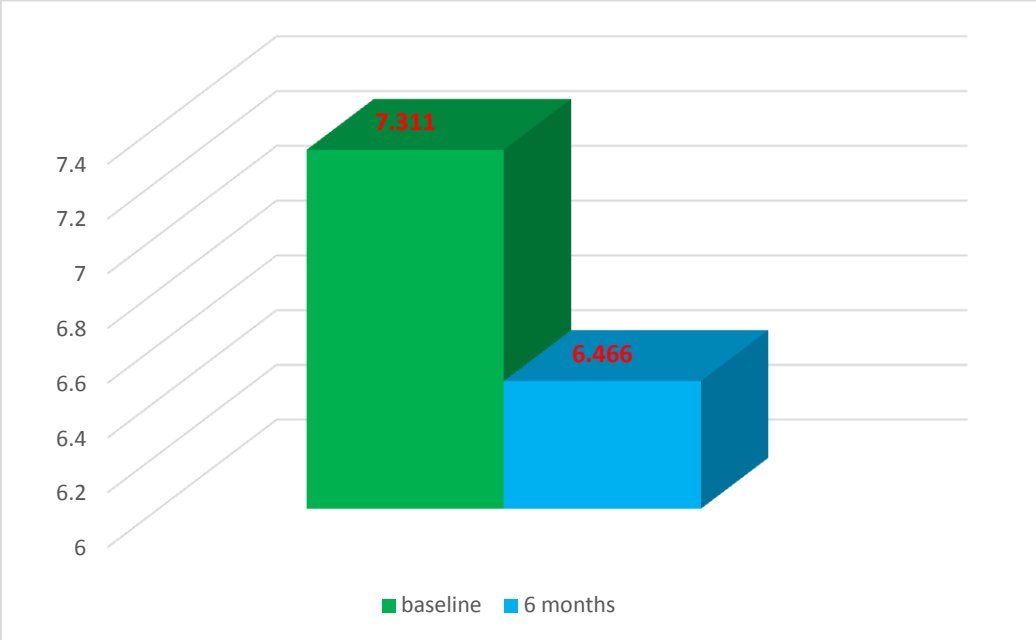
SI NO	Experimental site A		Experimental site B	
	BASELINE	6 MONTHS	BASELINE	6 MONTHS
1	5.26	4.41	4.85	3.92
2	9.01	7.72	4.97	3.33
3	9.29	7.87	6.48	5.56
4	5.97	5.44	7.95	6.12
5	7.39	6.92	4.17	3.69
6	6.19	5.85	6.83	5.77
7	6.64	5.18	8.54	7.79
8	7.76	7.61	8.08	7.24
9	3.6	2.84	2.85	1.97
10	11.8	10.82	13.4	12.1

Table 10: Mean difference in the values of CEJ – base of the defect experimental site A and experimental site B.

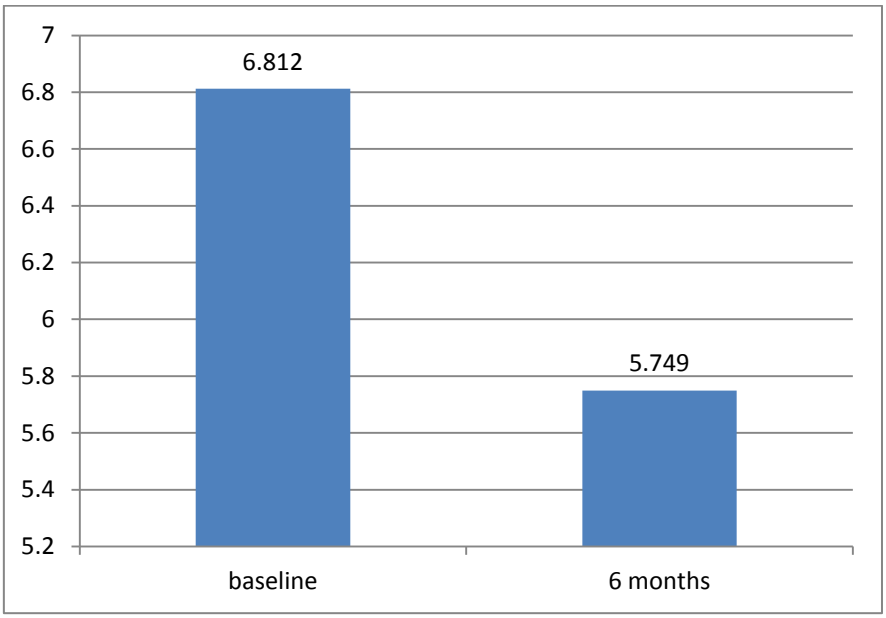
Time interval	Experimental site A			Experimental site B			A vs B	
	Mean CEJ-B ± SD (in mm)	Difference from baseline (in mm)	p value	Mean CEJ-B ± SD (in mm)	Difference from baseline (in mm)	p value	Mean difference (in mm)	p value
Baseline	7.311 ± 2.330			6.812 ± 2.969				
6 months	6.466 ± 2.223	0.845 ± 0.431	**0.000 HS**	5.749 ± 2.879	1.063 ± 0.413	**0.000 HS**	0.500 ± 1.269	**0.542** NS

p-Value = > 0.005 (not significant).

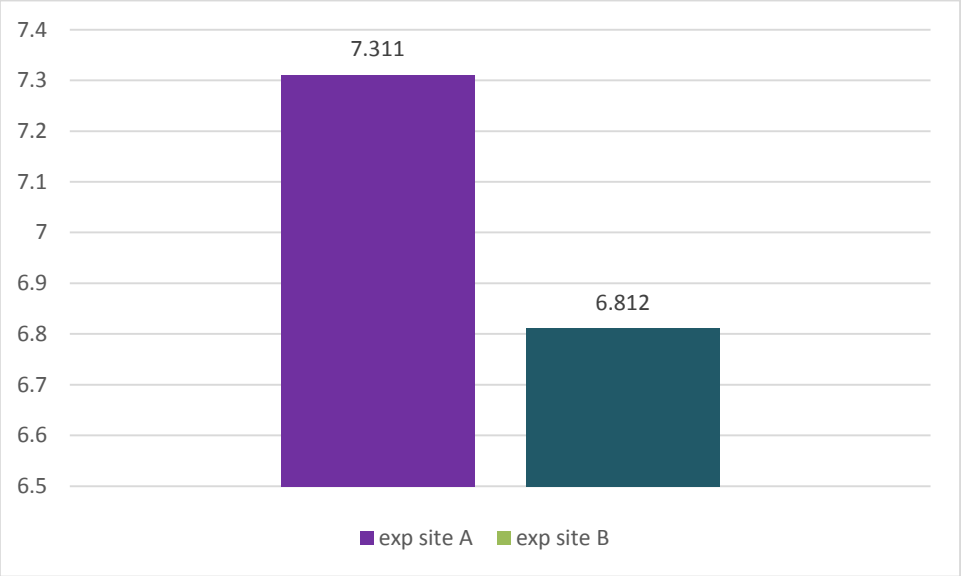
Graph 7: Comparison of cementoenamel junction to base of the defect in experimental site A (baseline Vs 6 months)



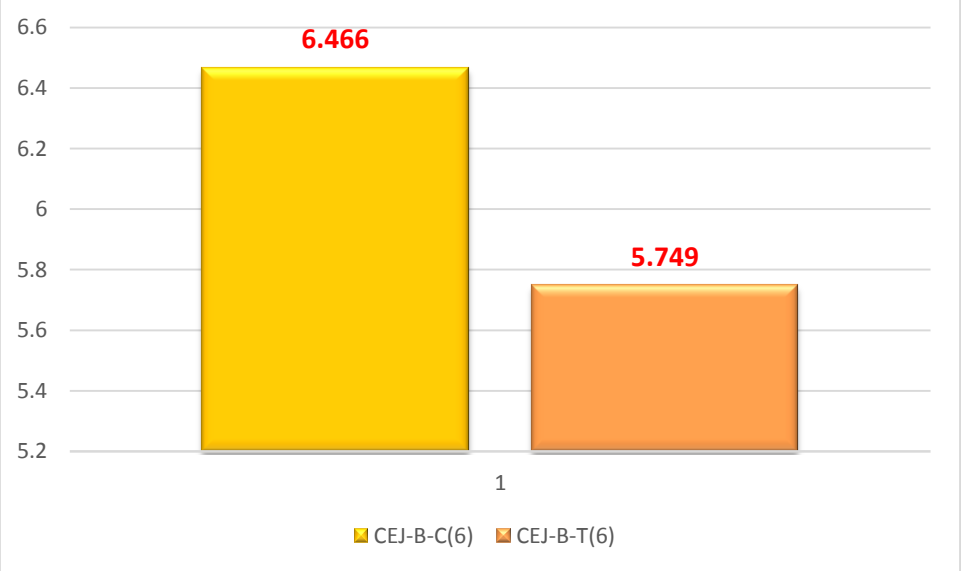
Graph 8: Comparison of cementoenamel junction to base of the defect in experimental site B (baseline Vs 6 months)



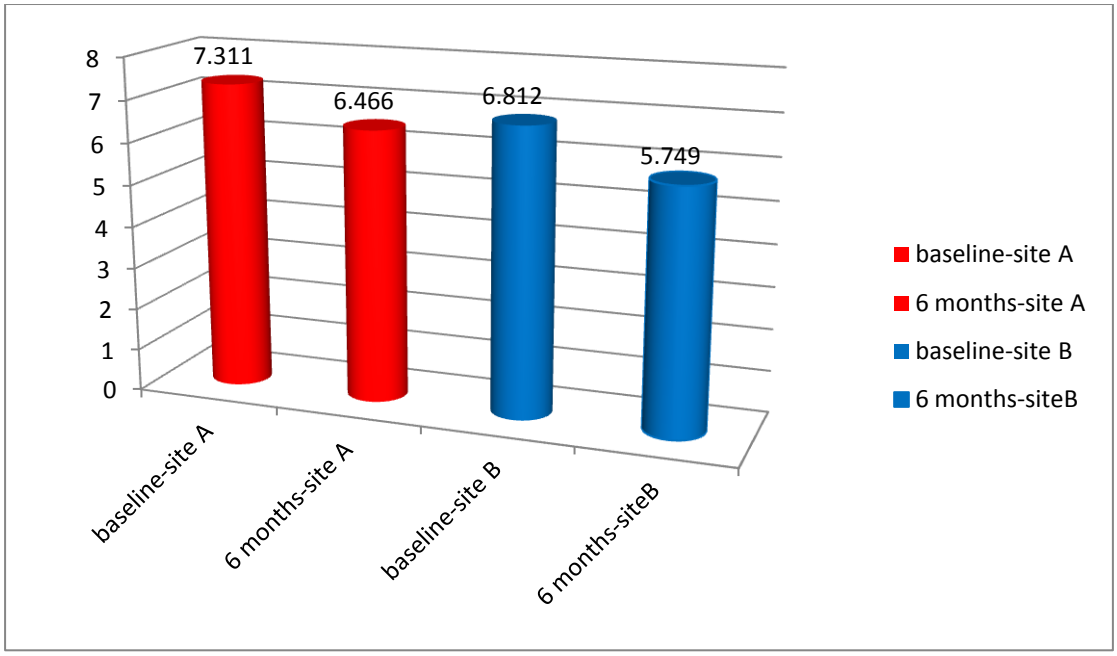
Graph 9: Comparison of cementoenamel junction to base of the defect at base line (Experimental site A Vs Experimental site B)



Graph 10: Comparison of cementoenamel junction to base of the defect at 6 months (Experimental site A Vs Experimental site B)



Graph 11: Comparison of CEJ to base of the defect (Baseline & 6 months in Experimental site A and Experimental site B)



Discussion

Discussion

Periodontal therapy is directed at arresting the progression of inflammatory periodontal disease with the goal of stabilizing the long term prognosis of the periodontium.[77] Surgical periodontal procedures are in general use for treating periodontal disease and the fact is that "diseased" roots can be cleaned more efficiently by "visual instrumentation" and hence it is the reason why surgical therapy is successful.[78] In case of advanced periodontal destruction, optimal treatment should involve not only controlling further periodontal infection but also regarding lost periodontium.[79] Regeneration of tissues has long been the altruistic goal of periodontal therapy.[80]

Periodontal regeneration means healing after periodontal surgery that results in the restoration of the attachment apparatus, namely, cementum, alveolar bone and periodontal ligament.[13] The objective of regeneration procedures is the formation of new cementum, periodontal ligament & alveolar bone adjacent to a previously pathologically exposed root surface.[77]

It is difficult to achieve full regeneration after traditional therapy because of different healing abilities among periodontal tissues. Nowadays, periodontal regeneration has become one of the goals of periodontal therapy.

The regeneration of any type of tissue is a complex biological process, requiring intricately regulated interactions between cells, locally acting growth factors, systemic hormones and growth factors & the extracellular matrix components in which these entities interact. Various biological approaches like use of growth factors & differentiation factors, application of extracellular matrix proteins and attachment of

extracellular matrix proteins and attachment factors and use of mediators of bone metabolism.[81]

The multifactorial process of periodontal regeneration requires an orchestrated sequence of biological events including cell adhesion, migration, multiplication and differentiation.

Recently, use of polypeptide growth factors gives promising results to promote periodontal regeneration. Growth factors are a group of naturally occurring proteins exhibiting varied potent local properties and they are the key regulators of biological events such as migration, attachment and proliferation of nearly all cell types. Hence, they play an important role in wound healing and regeneration of tissues.[5]

Choukroun's PRF (platelet-rich fibrin) may be considered as a second-generation platelet concentrate and it forms a strong natural fibrin matrix, which concentrates almost all the platelets and leucocytes of the blood harvest and shows a complex architecture as a healing matrix, including mechanical properties no other platelet concentrate offers. Large quantity of growth factors that enhance cell proliferation (especially platelet-derived growth factor [PDGF], matrix remodelling (especially transforming growth factor β [TGF β], and protection of the healing cells (anti-apoptotic role of insulin-like growth factor [IGF] are released during degranulation of platelets.[82]

Studies shows that PRF can release growth factors gradually and keep their activity to a relatively long period compared with PRP (platelet rich plasma) which is the first generation platelet concentrate. PRF has been demonstrated to release growth factors in a long term. Levels of released TGF- β1 and PDGF-AB markedly increased and

reached the highest amount at day 14, then decreased mildly, whereas PRP experienced only short term release of TGF- β1 and PDGF-AB, which reached the highest amount at 1 day and then decreased rapidly. PRF released maximum amount of TGF- β1 at 14 days, which may lead to maximum mineralization. The fibrin(ogen) content of PRFs may also contribute to the increased mineralization and hence PRF seems to be one of the most promising methods to enhance bone healing in a controllable, and relatively long-term effect, way.[83]

Commonly, dental lasers have been used for soft tissue surgical procedures. In recent years, many studies have been conducted to support the effectiveness of dental lasers as an adjunct to regenerative surgical procedures.

Diode lasers have been shown to have bactericidal effect, reduce inflammation and supported healing of periodontal pockets through the elimination of bacteria. Diode laser was proved to improve the gingival index, decrease probing pocket depth, bleeding on probing, bacterial content of periodontal pockets and improve the overall health of the periodontium. [84,85]

Diodes proved to have a stimulative/regulative effect on tissue that encompasses pain relief and wound healing and also Gallium-aluminum-arsenide (GaAlAs) diode have shown faster wound healing and bone formation after tooth extraction compared with unlased cases.

GaAlAs diode devices produced nonthermal bioactive reactions in the irradiated bone defect and underlying marrow which results in earlier osteogenesis. Low level laser therapy (LLLT) stimulates undifferentiated mesenchymal cells into osteoblasts, resulting

in increased osteogenesis and the increased blood circulation after LLLT might also bring a better supply of inorganic salts, promoting better bone formation.[22]

Each of the materials and techniques addresses specific aspects of the regeneration process. The combination of one or more the techniques currently available for periodontal regeneration has therefore the potential to enhance clinical results as compared to any of the techniques used alone.

Therefore, the purpose of the present clinico radiographic study was to evaluate efficacy of platelet rich fibrin assisted with and without laser application in the treatment of periodontal intrabony defects.

In this study, we have used platelet rich fibrin which is a second generation platelet concentrate. Ten patients with bilateral intrabony defects were included in this study and the selection of the sites for treatment had a probing depth of ≥ 5mm with radiographic evidence of vertical / angular bone loss. A total of 20 sites with intrabony defects from 10 patients were enrolled in this study. In order to avoid bias we have randomized the sited by coin toss method.

The split mouth design as adopted in the present study excludes the influence of patient-specific characteristics and facilitates a direct comparison of two groups.[86] It also facilitated the interpretation of trials by minimizing the effects of inter-patient variability.

In this study, the experimental site A were treated with open flap debridement and platelet rich fibrin placement and the experimental site B is treated with laser disinfection and platelet rich fibrin placement.

Clinical Parameters:

In the present study only those patients were considered for periodontal surgery who showed good oral hygiene maintenance during the Phase-I therapy. Oral hygiene status was assessed by taking the plaque index at baseline and then 1 month, 3 months and 6 months post-surgically. Plaque scores showed consistent reductions over the 6 months period.

The mean plaque index score at baseline was 1.43 ± 0.09 which was reduced to 0.10 ± 0.05 at 1^{st} month, 0.04 ± 0.02 at 3^{rd} month and 0.02 ± 0.02 at 6^{th} month showing a mean reduction of 1.3 ±0.10 at 1^{st} month, 1.38 ± 0.08 at 3^{rd} month and 1.4 ± 0.08 at 6^{th} month respectively, which were statistically highly significant with the p value of 0.000 ($p < 0.001$).

The gingival consistency is another critical factor for decision making in periodontal therapy. During the course of Phase-I therapy removal of plaque and other local irritants reduces the gingival inflammation resulting into tissue shrinkage and attaining the surgical manageability of gingiva.

The mean gingival index score at baseline was 1.03 ± 0.30 which was reduced to 0.08 ± 0.06 at 1^{st} month, 0.03 ± 0.02 at 3^{rd} month, 0.03 ± 0.02 at 6^{th} month showing a reduction of 0.9 ± 0.2 at 1^{st} month, 1.0 ± 0.3 at 3^{rd} month and 1.0 ± 0.3 at 6^{th} month, which were statistically highly significant with the p value of 0.000 ($p < 0.001$).

The mean probing pocket depth at baseline in experimental site A was 8.4 ± 1.5 mm which was reduced to 4.3 ± 0.9 mm at 6^{th} month showing a mean reduction of 4.1 ± 0.9 mm which was highly significant with the p value of 0.000 ($p < 0.001$) and the mean

probing pocket depth at baseline in experimental site B was 8.3 ± 2.5 mm which was reduced to 3.6 ± 1.1 mm showing a mean reduction of 4.7 ± 1.6 mm which was highly significant with the p value of 0.000 ($p < 0.001$).

Comparison between the two experimental sites revealed that the PPD reduction at 6 months post-operatively was slightly higher in sites treated with laser and platelet rich fibrin than those treated with platelet rich fibrin alone.

Comparison of two experimental sites revealed that the CAL gain at 6 months was more for experimental site B than the experimental site A, though the mean difference was statistically not significant. The gain in the clinical attachment level was thought to represent resolution of tissue inflammation, reformation of collagen fibers, new attachment to the root surface and the bone fill.

Radiographic Parameters:

The mean distance from CEJ to the base of the defect in experimental site A at baseline was 7.3 ± 2.3 mm which was reduced to 6.4 ± 2.2mm by the 6^{th} month which was statistically highly significant with the p value of 0.000 ($p < 0.001$). The mean distance from CEJ to the base of the defect in experimental site B at baseline was 6.8 ± 2.9 mm which was reduced to 5.7 ± 2.8 mm by the 6^{th} month which was statistically highly significant with the p value of 0.000 ($p < 0.001$).

Comparison between the two experimental sites revealed that the difference of 0.72 mm at 6 months was statistically not significant with the p value of 0.542 ($p > 0.05$).

We observed a significant improvement in all the clinical parameters such as plaque index, gingival index, probing pocket depth and mean defect fill. Similar improvements were observed by **Throat et al (2011)**[32] and **Sharma et al. (2011)**[33] in treating intrabony defects. The above studies was a clear evidence to the regenerative potential of platelet rich fibrin.

Study by **Chang et al. (2011)**[34] proved that the use of PRF is an effective modality for periodontal regeneration in intrabony defects. He also demonstrated the enhancement of p-ERK, OPG, and ALP expression by PRF. The result of the present study is in accordance with findings of the studies done by **Ajwani et al. (2015)**[61], where they observed a significant improvement in clinical and radiographic parameters in intrabony defects treated with platelet rich fibrin. Similar improvements were also observed by **Pradeep et al. (2012)**[38] and **Shah et al. (2014)**[53] in treating intrabony defects. Whereas, **Turkal et al. (2016)**[69] compared enamel matrix derivative with platelet rich fibrin in the treatment of intrabony defects and concluded that PRF did not improve both clinical and radiographic parameters and the result was contrary to the present study.

Study by **Alireza Fallah (2010)**[31] by using diode laser gave similar improvements in the clinical parameters and this is in accordance with present study. Similar improvements in clinical parameters were observed in studies done by **Davoud zare et al.(2014)**[56], **Tanya et al. (2015)**[67] and **Santhosh Dixit et al. (2016)**[71]. Study by **Moritz et al (1998)**[24], **Mirjana Gojkov-Vukelic et al. (2013)**[50] and **Mahitab et al (2014)**[57], proved the bactericidal property of diode laser. **Alice Dias Petri et al. (2010)**[30], **Gabriela et al. (2015)**[66] used Low level laser therapy and concluded that it is useful in bone regeneration by stimulating osteoblasts. Considering the fore mentioned regenerative potential of

platelet rich fibrin and diode laser we treated experimental site B with PRF and diode laser. Six months post surgery evaluation showed that significant improvement in all the clinical parameters but there was no statistically significant difference in the radiographic parameters among two groups. We could not find any published literature till date that evaluated the combined effect of PRF and diode laser. Comparison between the sites treated with PRF alone and PRF with diode laser at the end of 6 months showed statistically significant difference in clinical parameters and the radiographic assessment showed it is not statistically significant.

Though surgical re-entry or histologic evaluation is considered to be gold standard to assess true periodontal regeneration, we are unable to perform because it causes a degree of ethical concern.

Further studies with a longer duration, large sample size, better imaging techniques, Histologic evaluation must be undertaken, to determine the beneficial effects of the adjunctive use of PRF with Diode Laser, before arriving into a definitive conclusion.

Summary and Conclusions

SUMMARY AND CONCLUSION

The present study was conducted to evaluate clinically and radiographically, the efficacy of platelet rich fibrin assisted with and without laser application in the treatment of periodontal infrabony defects and their effects on the various clinical parameters like, Plaque Index, Gingival Index, Probing Pocket depth and Clinical attachment level. A total of twenty intrabony defects in ten patients were treated in a split mouth design. Experimental site A were treated with open flap debridement and platelet rich fibrin placement and Experimental site B were treated with Laser disinfection and platelet rich fibrin placement. Clinical parameters like Plaque Index, Gingival Index were recorded at baseline, 1 month, 3 months and 6 months. However, probing pocket depth, clinical attachment level and radiographic assessment were recorded only at baseline and 6 months.

From the results of the study we arrive at the following conclusions:

- There was a significant reduction in plaque index, gingival index, probing pocket depth in both the experimental sites over a six months time period.
- Platelet rich fibrin is clinically effective in the treatment of intrabony defects.
- Both groups showed the potential of enhancing the periodontal regeneration, however, there is no significant difference in the radiographic parameters between the monotherapy of platelet rich fibrin and a combination therapy of platelet rich fibrin and diode laser.
- Both the materials (PRF and laser) were safe to use, without causing any immunologic or antigenic reactions in any of the patients.

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Annexure

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ANNEXURE-I

Ref:UT:BDSC:IRB-EC/2014 Date:18.11.2014

From

Institutional Review Board-Ethical committee,
Best dental science college,
Madurai.

To

The Controller of Examinations,
The Tamil Nadu DR.MGR Medical University,
No. 69, Anna salai,
Guindy,
Chennai-600 032

Sir/Madam

The Dissertation topic titled "EFFICACY OF PLATELET RICH FIBRIN ASSISTED WITH AND WITHOUT LASER APPLICATION IN THE TREATMENT OF PERIODONTAL INTRABONY DEFECTS – A CLINICO RADIOGRAPHIC STUDY" submitted by Dr.S.P.BRINDHA DEVI postgraduate student has been approved by Institutional Review Board of Best Dental Science College on 18.11.2014.

DR.K.S PREM KUMAR.M.D.S.,
VICE PRINCIPAL
MEMBER SECRETARY
INSTITUITONAL REVIEW BOARD-ETHICAL COMMITTEE
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MADURAI

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ANNEXURE 2

INFORMATION SHEET

We are conducting a study on *"***EFFICACY OF PLATELET RICH FIBRIN ASSISTED WITH AND WITHOUT LASER APPLICATION IN THE TREATMENT OF PERIODONTAL INTRABONY DEFECTS – A CLINICO RADIOGRAPHIC STUDY".** The identity of the patients participating in the research will be kept confidential throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Taking part in the study is voluntary. You are free to decide whether to participate in the study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Name of the patient Signature / Thumb impression

Name of the investigator Signature

Date

ANNEXURE 3

INFORMED CONSENT FORM

Efficacy Of Platelet Rich Fibrin Assisted With And Without Laser Application In The Treatment Of Periodontal Intrabony Defects – A Clinico Radiographic Study

Name: Age/Sex Op.No: Date:

Address:

I, _____ aged _____ have been informed about my role in the study.

1. I agree to give my personal details like name, age, sex, address, previous dental history & the details required for the study to the best of my knowledge.

2. I will co-operate with the dentist for my intra oral examination & extra oral examination.

3. I will follow the instructions given to me by the doctor during study.

4. I permit the dentist to take blood sample, photos, intraoral radiographs & I accept to undergo bone regenerative procedures as required for the study.

5. If unable to participate into study for reasons unknown, I can withdraw from the study.

In my full consciousness & presence of mind, after understanding all the procedures in my own language, I am willing & give my consent to participate in this study.

Name of the patient: Name of the investigator:

Signature/Thumb impression Signature

ஆராய்ச்சி ஒப்புதல் கடிதம்

பெயர் :

தேதி :

வயது :

புறநோயாளி எண் :

பாலினம் :

ஆராய்ச்சி சேர்க்கை எண்:

கீழ்க்காணும் நிபந்தனைகளுக்கு நான் ஒப்புதல் அளிக்கிறேன்

1. என் பெயர், வயது, பாலினம் , முகவரி, பல் சம்மந்தப்பட்ட சிகிச்சை மற்றும் என்னுடைய முழு விவரத்தினை கொடுக்க நான் முழு மனதுடன் ஒப்புக் கொள்கிறேன்
2. என்னுடைய வாயின் உள்பகுதி (அல்லது) வெளிபகுதியை மருத்துவர் பரிசோதனை செய்ய ஒத்துழைக்கிறேன்.
3. நான் மருத்துவர் அளிக்கும் விதிமுறைகளை தவறாமல் கடைபிடிப்பேன்.
4. மேற்கண்ட ஆராய்ச்சிக்காக என் இரத்தம, புகைப்படம், பற்கள் சம்பந்தப்பட்ட எக்ஸ்ரே மற்றும் ஈறு அறுவை சிகிச்சை எடுக்க மருத்துவருக்கு அனுமதி அளிக்கிறேன்.
5. நான் மேற்கண்ட ஆராய்ச்சியில் பங்குபெற முடியவில்லை என்றால் ஆராய்ச்சியில் இருந்து விலகிக் கொள்வேன்.

மருத்துவரின் ஆராய்ச்சி சம்பந்தப்பட்ட விவரங்களை முழுமையாக புரிந்து கொண்ட பிறகு, என் முழு மனதுடனும் மற்றும் சுய நினைவுடனும் இந்த மருத்துவ ஆராய்ச்சியில் பங்கு கொள்ள சம்மதிக்கிறேன்.

நோயாளியின் பெயர் கையொப்பம்

ஆராய்ச்சியாளரின் பெயர் கையொப்பம்

ANNEXURE 4

EFFICACY OF PLATELET RICH FIBRIN ASSISTED WITH AND WITHOUT LASER APPLICATION IN THE TREATMENT OF PERIODONTAL INTRABONY DEFECTS – A CLINICO RADIOGRAPHIC STUDY

CLINICAL PROFORMA

Op No: **Sl. No:**

Date:

Name: **Age/ Sex:**

Address: **Occupation:**

Chief Complaint:

Medical history:

Dental history:

Family history:

Personal History:

CLINICAL PARAMETERS – AT BASELINE Date:

PLAQUE INDEX (LOE 1967)

	17	16	15	14	13	12	11	21	22	23	24	25	26	27
BUCCAL														
PALATAL														
BUCCAL														
LINGUAL														
	47	46	45	44	43	42	41	31	32	33	34	35	36	37

PI SCORE:

GINGIVAL INDEX (LOE 1967)

	17	16	15	14	13	12	11	21	22	23	24	25	26	27
BUCCAL														
PALATAL														
BUCCAL														
LINGUAL														
	47	46	45	44	43	42	41	31	32	33	34	35	36	37

PROBING DEPTH & CLINICAL ATTACHMENT LEVEL

TOOTH NUMBER		PROBING POCKRT DEPTH	CLINICAL ATTACHMENT LEVEL
TEST			
CONTROL			

SIGNATURE OF THE GUIDE:

CLINICAL PARAMETERS – AT 1 MONTH Date:

PLAQUE INDEX (LOE 1967)

	17	16	15	14	13	12	11	21	22	23	24	25	26	27
BUCCAL														
PALATAL														
BUCCAL														
LINGUAL														
	47	46	45	44	43	42	41	31	32	33	34	35	36	37

PI SCORE:

GINGIVAL INDEX (LOE 1967)

	17	16	15	14	13	12	11	21	22	23	24	25	26	27
BUCCAL														
PALATAL														
BUCCAL														
LINGUAL														
	47	46	45	44	43	42	41	31	32	33	34	35	36	37

CLINICAL PARAMETERS – AT 3 MONTHS Date:

PLAQUE INDEX (LOE 1967)

	17	16	15	14	13	12	11	21	22	23	24	25	26	27
BUCCAL														
PALATAL														
BUCCAL														
LINGUAL														
	47	46	45	44	43	42	41	31	32	33	34	35	36	37

PI SCORE:

GINGIVAL INDEX (LOE 1967)

	17	16	15	14	13	12	11	21	22	23	24	25	26	27
BUCCAL														
PALATAL														
BUCCAL														
LINGUAL														
	47	46	45	44	43	42	41	31	32	33	34	35	36	37

CLINICAL PARAMETERS – AT 6 MONTHS Date:

PLAQUE INDEX (LOE 1967)

	17	16	15	14	13	12	11	21	22	23	24	25	26	27
BUCCAL														
PALATAL														
BUCCAL														
LINGUAL														
	47	46	45	44	43	42	41	31	32	33	34	35	36	37

PI SCORE:

GINGIVAL INDEX (LOE 1967)

	17	16	15	14	13	12	11	21	22	23	24	25	26	27
BUCCAL														
PALATAL														
BUCCAL														
LINGUAL														
	47	46	45	44	43	42	41	31	32	33	34	35	36	37

PROBING DEPTH & CLINICAL ATTACHMENT LEVEL

TOOTH NUMBER		PROBING POCKRT DEPTH	CLINICAL ATTACHMENT LEVEL
TEST			
CONTROL			

SIGNATURE OF THE GUIDE:

CLINICAL PARAMETERS

PARAMETERS	BASELINE	1 MONTH	3 MONTHS	6 MONTHS
PLAQUE INDEX				
GINGIVAL INDEX				
PROBING POCKET DEPTH				
CLINICAL ATTACHMENT LOSS				

SIGNATURE OF THE GUIDE: